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PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF ETHYLENE ON TULIPS

by

© MARY ANN KLYNE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled

PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS

OF ETHYLENE ON TULIPS

submitted by Mary Ann Klyne

in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

The purpose of this work was to study the morphological changes in tulips, especially the influence of ethylene on nucleic acid and protein synthesis.

At the concentrations of ethylene employed (less than 10 ppm), ethylene appeared to stimulate the synthesis of nucleic acids and proteins. The stimulation was dependent on the physiological state and age of the plant, as well as on the length of ethylene treatment and concentrations of ethylene employed. Similar results were obtained with both low concentrations of ethylene over an extended period, and brief exposures to high ethylene levels.

The susceptibility of tulip flowers to ethylene varied with the developmental stage of the flower; it was higher during periods of active metabolism (period of flower initiation and formation, and the period prior to blossoming of the bud). Severe blasting occurred when buds were exposed to ethylene concentrations of 0.3 ppm or higher, for periods of exposure longer than two days.

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LIST OF ABBREVIATIONS

CEPA	2-chloroethylphosphonic acid.
DNA	deoxyribonucleic acid.
IAA	indole acetic acid.
PCA	perchloric acid.
ppm	parts per million.
RNA	ribonucleic acid.
m-RNA	messenger ribonucleic acid.
t-RNA	transfer ribonucleic acid.
TCA	trichloroacetic acid.

I. INTRODUCTION

During the Dark Ages a limited number of flowering-bulb species was found in Western Europe. It was only in the 1600s that tulips (Tulipa gesneriana) appeared on the market. Carolus Clusius (1525-1609) in his writings described his findings and ideas on the morphology, taxonomy, propagation, genetics, senescence and the influence of environment on the growth of tulips. In addition, he described the colour break of the flowers (33) which indicated his concept of the pathological nature of this phenomenon.

Since the basic work on flower formation most investigations have centered around the influence of temperature on this process (12, 43, 59, 86). It should be emphasized, however, that there is almost no knowledge about the basic reactions within the bulbs as influenced by temperature and other environmental factors (96). Generally, the essential processes leading to flowering take place during the dry storage of the bulbs, but the conditions after planting are nonetheless as important. The understanding of these factors is still unclear and the basic process leading to flower abortion, blasting and necrosis is not fully understood. Recent investigations (72, 74, 78) have indicated that ethylene may be involved in these disturbances.

Blasting of flower-buds and inhibition of shoot growth under the influence of ethylene can be observed in tulip bulbs before planting, even when the roots have not yet developed (72). Bulbs, in the receptive stage, are sensitive to ethylene even at concentrations of 0.3 ppm. Such levels of the gas are sufficient to upset the natural metabolic processes of the bulbs, hence resulting in disorders (74, 78).

II. LITERATURE REVIEW

A. The Physiology of Tulips

The geophytic habit is wide spread in families of flowering plants (95). Although, it occurs most widely in the Liliceae, Amaryllidaceae and Iridaceae, it is also present in other families.

The bulb habit confers the benefits of vegetative reproduction by the development of lateral buds. According to Grainger's classification (39) bulb producing plants are 'indirect flowering plants' because of the interpolation of an annual 'rest period' into the life-history; floral initiation occuring during this period of minimum vegetative growth. Thus the life-history of the bulb falls into three stages:-

1. Storage of dry bulbs,
2. Growth to flowering,
3. Growth from flowering to death of the aerial parts and lifting.

1. Storage of Dry Bulbs

This is the most widely studied stage in the life-history of the bulb. Hartsema (42) suggested the following reasons for the storage of bulbs:-

- to produce early flowering, by low or high temperature treatment,
- to break dormancy, by the use of low or high temperature,
- to prevent flowering, by temperature controlled storage which results in larger bulbs the following season.

a. Bulb Size in Relation to Flowering

Bulbs below a certain size do not flower. It is not, however, the only factor determining flower formation; bulb weight is equally important. Minimum size of the bulb may be affected by other factors (43). The critical bulb size for flowering is between 6 and 9 cm, nevertheless,

some varietal differences do occur (85, 87).

b. Floral Initiation (Postharvest Maturation Period)

The tulip species do not have a true dormant period. Metabolic activity within the bulb is high during the period of apparent quiescence which follows lifting. It is during this period that the floral initials of next season's growth are laid down. In the Northern Hemisphere the embryonic flower is generally completely formed by early September. The developmental stages of the flower, as described by Blaauw, can be classified using the symbols designated by Beijer (43), Figure 1.

The optimum temperature for floral initiation is well defined. Data on tulips (59) indicate that the developmental stage A1 is most rapid within the range of 14 - 20 C. The temperature at which the flower increased in length fastest showed only a slight evidence of a shift from 17 C to below 15 C. This period of rapid floral growth coincides with the rapid elongation of the main flower-axis, and as in the case of the former is favoured within the same temperature range. Once the flower has been formed (G-stage, Figure 1) the bulb is ready to be cooled.

c. Low-Temperature Requirement for Rapid Flowering (Cooling Period)

The low-temperature requirement, following a high-temperature treatment after lifting, is in some ways analogous to vernalization; but the difference has been stressed by Chouard (27). In true vernalization a cold treatment is used to create the capacity for subsequent flowering, not to initiate flower-primodia (as in iris), or to promote rapid growth after floral initiation (as in tulip and narcissus).

A cold treatment is required after the floral initiation of the tulip is completed to promote rapid elongation of the aerial structures of next season's growth. The temperature and period of this cold treatment

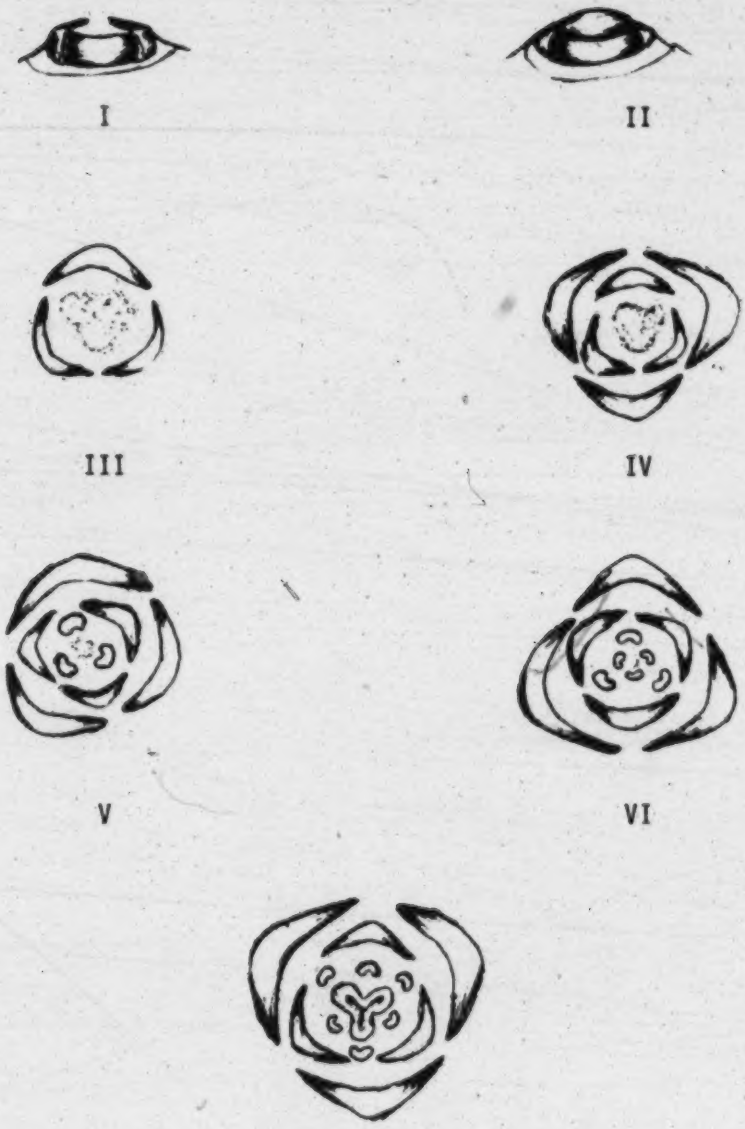


FIGURE 1: A Schematic Representation of Flower Formation of Tulipa gesneriana (43)

Stage I	scales and foliage leaves splitting off, apex of growing point still low and flat.
Stage II	apex of growing point dome-shaped.
Stage III (P1)	3 outer tepals distinguished as separate primodia.
Stage IV (P2)	3 inner tepals distinguished.
Stage V (A1)	first whorl of stamens visible.
Stage VI (A2)	second whorl of stamens visible.
Stage VII (G)	3 carpel-primodia visible as separate walls.

is dependent on the tulip cultivar. Rees (86) and others (102) have shown that for cultivar Paul Richter a cold treatment of 10 C for a period of twelve weeks is essential for optimum growth.

A possible relationship between the cooling period and the conversion of starch has been suggested (11). It was shown later (5) that low storage temperatures of the bulbs increased the sugar concentration in the leaves. These findings were not confirmed by others (50, 90, 91). In a recent study (65) it was suggested that low temperatures activate carbohydrate metabolizing enzymes, possibly via hormonal control.

d. Floral Abnormalities

These abnormalities are associated with extreme temperatures during storage. Exposures to these temperatures over an extended period resulted in more pronounced floral abnormalities.

At low temperatures a higher number of floral initials are laid down in the tulip bud (43); this does not occur at the expense of the leaves (11). Blaauw attributed this to the larger plant apex developed at low temperatures.

There is some evidence (87) that 'tired-leaf' (the last leaf on the tulip stem is partly petalloid and fused to the perianth parts) is affected by temperature. 'Tired-leaf' symptoms occurred with a lower frequency in bulbs stored at cooler temperatures during the postharvest maturation period.

e. Development of Vegetative Parts in Relation to Temperature

Although temperature during storage has no effect on the final number of leaves produced by the tulip (12), it influences the rate of leaf production. As the leaf-number is predetermined in the bulb, the time of floral initiation may be considered as being controlled by vegetative growth and hence under the direct control of temperature. The

rate of shoot growth is dependent on temperature, higher temperatures favouring shoot elongation once the leaves are visible.

In tulips rapid root development and main-axis elongation occur at the same optimum temperature; for cultivar Paul Richter it is at 10 C (86). Maximum root development, however, follows storage at 13 C (12).

2. Growth to Flowering (Forcing Period)

The optimum temperature for rapid flowering increases when the leaves become visible. For the cultivar Paul Richter it was shown that a day temperature of 18 C and a night temperature of 16 C were suitable for bringing the plants into flower (86, 102). The process of placing the plants in these temperatures should be gradual and may be achieved by stepwise increments of the temperature or by shading the plants before bring them to higher temperatures.

Hartsema (43) has stated that during this period neither light nor day length is important because flower formation in bulbous plants is not affected by either. She also stated that the flowering of tulips is unaffected by short day conditions or low light intensities.

3. Growth from Flowering to Lifting

The physiology of bulbous plants during this stage has received the least attention. Once the flowers have been harvested the market value is in the bulbs, and this is dependent on the number and size of the bulbs produced. Thus the market requirements dictate the spacing of the bulbs - large size bulbs being produced only under conditions of comparatively wide spacing.

There are two exhaustion periods during the growth of the tulip. The first occurs when the tops and flower stem elongate, after the cooling period, at the expense of the old bulb which then disintegrates.

The second occurs when the tops elongate rapidly during flower maturation. The greatest amount of new bulb growth occurs after the blooming of the flowers and it is during this period that fertilization should be high to ensure a good crop of bulbs for the following season (71).

The partitioning of dry weight increment in tulips between the main and secondary bulbs (giving extremes of a large number of small bulbs or a few large bulbs) may be controlled by temperature during storage. Mulder and Blaauw (70) found that larger bulbs were produced after storage at 20 C (7 weeks) followed by 17 C (4 weeks) as compared with a greater number of smaller bulbs produced after storage at 20 C for 11 weeks.

The stage of bulb 'ripeness' for lifting has been investigated. Early lifting of bulbs reduces the number that flower and the size of flowers produced (87). It also checks organ formation, but this may be offset by storing the bulbs at slightly higher temperatures than that normally recommended.

B. Effects of Ethylene

The effects on plant plant growth and leaf abscission of illuminating gas was the origin of the 'ethylene story'. Nevertheless, some confusion arose because carbon monoxide mimics, though at much higher concentrations, the effects of ethylene. Denny (32) was the first to show that ethylene hastened the coloration of citrus fruits.

From 1933 onwards a detailed investigation of the effect of ethylene on the respiration of apples and the behaviour of the stored fruit was carried out (54). In 1950 the work on the effect of ethylene on various fruits in storage was summarized by Porritt (83). Parallel with this work was the growing interest in the interrelationships between auxins and ethylene. In addition, there appeared evidence that the synthesis of

certain nucleic acids were influenced by both auxin (100) and ethylene (61).

For many years ethylene physiology was the province of those concerned with fruit ripening and aspects of postharvest physiology. Recent investigations (2, 15, 61, 90) revealed that the effects of ethylene encompass a much wider field. These studies have shown that ethylene regulated phenomena can be broadened to include:- breaking of dormancy, regulation of swelling and elongation, hypertrophy, induction of adventitious roots, epinasty, hook closure, inhibition of leaf expansion, control of flower induction, exudation, abscission and senescence (2).

1. Is Ethylene a Growth Hormone?

Research into the mechanism of action of ethylene has been active, but, the primary act of ethylene still remains to be solved. An immediate problem centers on the question of whether ethylene is a growth hormone. The first proof of the endogenous production of ethylene by plant tissues led to the suggestion in 1935 (30) that ethylene should be considered a plant hormone. In the last few years correlative evidence was obtained to support this suggestion (84).

Abeles (2) classifies ethylene as a 'stirone' (to stir up the cell) because directed transport is not an essential feature of ethylene action. The definition of a stirone is similar to that of a hormone except that stirones move from the site of production to the site of action by simple diffusion. Stirones are simple organic molecules and are effective in small quantities. They cause recognizable physiological phenomena and are not consumed during the course of action.

2. Auxin and Ethylene Interrelationships

The earlier proposal (112) that certain effects of auxin were attributable to their stimulation of ethylene production has been reiterated in recent studies. Thus many of the effects attributed to concentrations

of auxin higher than those promoting optimum growth were shown to be caused by ethylene (18, 19, 20, 22, 26, 36, 70, 93).

a. Leaf Abscission

Gawadi and Avery (37) advanced the theory that the auxin-ethylene balance regulates abscission. Hall (40) supported this theory and demonstrated that it was not the absolute amount of IAA or ethylene *per se* which was the important factor; rather it was the relative balance between auxin and ethylene concentrations. It was later argued (16) that abscission occurs in two stages: an inductive stage and an operative stage; ethylene apparently acting during the latter phase.

Experiments by Burg (16), in which whole plants were exposed to ethylene, showed that as leaves age less ethylene is required to cause abscission. He inferred from this that ethylene was responsible for the reduction in the amount of diffusable auxins present in intact plants and suggested that in some plants ethylene may interfere with the polar transport of auxin. Morgan *et al* (66) demonstrated the enhancement of IAA-oxidase activity by ethylene, thus suggesting that the gas may cause auxin destruction. Finally, Burg put forward the proposal that ethylene may also function by retarding auxin synthesis (104).

b. Growth: (i) Roots

As early as 1935 Zimmerman and Wilcoxon (112) showed that IAA and ethylene caused the initiation of adventitious roots on stems and leaves. However, the situation was confused because although auxin and ethylene caused the initiation of adventitious roots, both auxin and ethylene also caused inhibited growth of root sections (pea). With the introduction of the new, sensitive gas-liquid chromatography methods of ethylene determination (26), it was shown that all the pea root sections produced little ethylene, in the presence of 0.1 mM IAA substantial amounts of

ethylene were produced. It was this IAA induced ethylene that inhibited the root growth. This substantiated the theory that auxin inhibited root growth by inducing the production of ethylene.

Andreae et al (6) objected to the above theory. They showed that the inhibitory action of IAA on the growth of excised pea root sections was fully reversible on transfere of the root sections to IAA-free solutions. In contrast, inhibition by ethylene was totally irreversible. In addition, IAA inhibited the growth from zero time while ethylene was generally without effect during the first three to six hours. From these differences they concluded that it was unlikely that ethylene could play more than a minor role in mediating inhibition of pea root growth by IAA.

b. Growth: (ii) Shoots and Whole Seedlings

Studies (19) on the effect of IAA and ethylene on etiolated shoot sections revealed that IAA effects were dependent on the IAA-induced ethylene production. Light grown (green) pea seedlings were much less sensitive to ethylene and responded less to exogenous IAA in ethylene production (19). The green tissue swelled as a result of endogenous ethylene production; however, this IAA-induced ethylene was never sufficient to offset the auxin-induced increase in elongation (19). Holm and Abeles (47) found that soybean seedlings were less sensitive to ethylene than the pea seedlings used by Burg (19); from this study they inferred that in some plants a balance between endogenous ethylene and carbon dioxide could regulate growth.

c. Apical Dominance

In studies on the effects of ethylene on auxin transport in stem sections, Morgan and Gausman (67) showed that the effect of ethylene on the distribution of adsorbed auxin was complex. Both uptake and transport of auxin were affected, but the effect on transport from apical segments to basal segments was real and not merely due to reduced uptake. In these

studies the destruction of auxin did not appear significant. The inhibition of polar transport of auxin by exogenous ethylene could lead to local surpluses or shortages of auxin. In areas of surplus, production of endogenous ethylene accelerated by auxin could lead again to shortages in certain areas. This could, for example, lead to the breaking of apical dominance which is normally maintained by a high auxin concentration in terminal buds. This interrelationships was supported by Burg and Burg (21) who showed Tater (100) that auxin induced ethylene formation could account for the inhibiting action of IAA on bud growth.

The fact that ethylene actually induced the inhibition of bud growth caused by (added) IAA did not solve the question of the role of auxin in apical dominance - the amount of auxin required was more than that found naturally in the stem. All that the study showed was that apical dominance induced by applied auxin was mediated through the ethylene produced.

3. Regulation of Nucleic Acid and Protein Metabolism

Plant and animal hormones affect nucleic acid metabolism in a wide variety of systems. There is an increasing amount of literature which relates hormonal regulation to nucleic acid metabolism (53).

Skoog (98) was the first to propose that auxin levels affected the DNA:RNA ratio. This then apparently influenced the relative rate of cell multiplication and cell enlargement. Auxin concentrations have a pronounced effect on nucleic acid metabolism. At low concentrations auxins favour DNA synthesis and cell division, while at slightly higher concentrations RNA synthesis and cell enlargement are favoured; however, still higher auxin concentrations block both nucleic acid metabolism and cell enlargement (98).

Ethylene may effect auxin levels by any of the following mechanisms:-

a. destruction of auxin (66),

b. retardation of auxin synthesis (102),

c. interfering in the transport of auxin (10, 21).

Therefore, ethylene may alter the synthesis or content of nucleic acids either directly or indirectly (through its action on auxin). Turkova et al (101), in their study on ethylene-induced epinasty of tomato leaves, presented evidence which showed that ethylene caused the alterantion in the DNA:RNA ratio in the petioles of these leaves.

Plant growth is either promoted, inhibited or unaffected by ethylene depending upon the kind of tissue involved. Holm and Abeles (46) reported that the DNA content in bean leaves was unaffected by a 7-hour exposure to ethylene, whereas soybean seedlings treated with ethylene stopped synthesizing DNA in the apex (where growth was inhibited) and promoted DNA synthesis in the subapical part (where swelling took place), (47). Burg and Burg (22) found that a similar situation existed in pea seedlings.

Ethylene caused an increase in RNA synthesis in the abscission layer, and also in preclimacteric fruit (3, 46, 108). The increase occurred in all classes of RNA and preceded that of protein synthesis (3, 48). Nearest neighbour analysis (46, 47), of the RNA synthesized, demonstrated that there was a qualitative difference between the RNA synthesized by the normal tissue and that treated with ethylene. From this it may be inferred that ethylene regulated RNA synthesis and caused a change in both the quality and quantity of the RNA formed.

Increases in protein synthesis during ripening, abscission or after fumigation of vegetative tissue have been observed. Kidd and West (54) reported an increase in protein content in ripening pears and apples; this was subsequently confirmed by others (48). Increased protein syntheses were also observed in other fruits, however, Sacher and Salminen (93), who failed to observe an increase in the RNA levels in preclimacteric bananas

or avocados treated with ethylene, failed to observe increased protein syntheses in these fruits.

Histochemical staining and radioautography have substantiated that increased protein synthesis occurs during abscission. Webster (108) presented clear and vivid microphotographs showing enhanced protein synthesis in the separation layer of bean tissue. Valdovinos (105) demonstrated that endoplasmic reticulum of tobacco flower pedicels undergoing abscission was more pronounced than in intact controls.

Ethylene has also been found to increase protein levels in vegetative tissue. Elmer (35) reported that ethylene enhanced the protein content of potato sprouts. Soybean seedlings, fumigated with ethylene, were shown to contain greater quantities of protein in the basal and elongating parts, but not in the apical regions where growth was inhibited (47).

4. Effect of Ethylene on Tuber and Bulb Initiation

Tuber initiation in Solanum species involves a lateral expansion of cells in sub-apical regions of the stolon (13). When the stolons were treated with ethylene in light tight boxes, ethylene inhibited the extension growth of both sprout and stolon. The positive geotropic response of the shoot was lost and root development inhibited; however, the most striking response was the swelling of all rapidily expanding regions (sub-apical regions of stolons, stems and axillary buds). The sub-apical swellings on these stolons were similar in morphology and anatomy to normal tubers at a similar stage of development. In contrast to tuber initials, these 'swellings' lacked starch (25).

Brian et al (14) showed that although growth inhibitors increased tuberization of Dahlias, tuber growth was always better under short day conditions. They found that when Dahlia plants (untreated) were exposed to short day conditions greater quantities of ethylene were evolved

within the second and third week after the start of the short day conditions. The rate of ethylene evolution decreased during the period of tuberization. This sudden increase in ethylene production appeared to be related to the induction or initiation of the tubers, and is in agreement with earlier work on tuber initiation in Solanum (35). During the later stages of tuber promotion and growth, where mobilization of assimilates was involved, ethylene inhibited tuberization (14).

As in the case of tuberization of Dahlias, bulbing of onions is induced by photoperiodism. The initiation of bulbing is indicated by swelling of leaf-bases and increase in cell size, which is then accompanied by translocation of assimilates to these tissues (44, 60). It was shown by Levy and Kedar (60) that when onion plants (in the fourth true leaf stage) were treated with varying concentrations of Ethrel during non-inductive day lengths (56), the ethylene released (from Ethrel) within the plant caused bulb initiation. It was shown that the higher concentrations of Ethrel were more effective in causing early bulb initiation and increased rate of bulbing. Nevertheless, leaf and bulb growth were retarded (56). IAA (28) has been shown to cause swelling of leaf-bases of onion, and since auxin can induce ethylene formation (1, 15); ethylene may well be the factor responsible for bulb initiation.

5. Some Disorders in Tulips Caused by Ethylene

Disorders of tulips may appear in a variety of forms. The cause (or causes) of these disorders, with the exception of flower break (33) remains unknown. Two of the more serious disorders in tulips, which lead to either disturbance or complete failure of flowering are:- bud necrosis and flower-bud blasting. In both disorders the external appearance of the bulb is completely normal; internally, the buds are affected.

a. Bud Necrosis

The term 'bud necrosis' is applied to those aberrations in which the generative main bud of the bulb decays from within. This decay is accompanied by a brownish-black discoloration, the affected organs usually being wet. Depending on the extent of the decay over the various organs, plants developing from affected bulbs will be defective to corresponding degrees (77).

Preliminary investigations (51) yielded indications that volatile substances were involved in bud necrosis, and the extensive studies which followed showed that at least two conditions have to be satisfied before this disorder could materialize (73). These were the presence of ethylene and of mites.

Ethylene causes aberrant bud development in the period during and shortly after flower formation, the characteristic feature being that the buds are opened at the tip, normal buds being completely closed (Figure 2). The occurrence of open buds is related to more rapid growth of the stamens than that of the young leaves, as a result of differences in growth retardation caused by ethylene. In cases of severe aberrations the floral organs may even project above the young leaves (Figure 2). A concentration of ethylene less than 0.3 ppm is the threshold value for open bud formation (73).

The decay associated with bud necrosis is not caused by ethylene, but by mites which gain access to the bud through the opening at the tip of the foliage leaves. Thus, although ethylene is not responsible for the decay, it is, nevertheless, the factor responsible for bud necrosis. Bud necrosis almost always coincides with the occurrence of open buds; this disorder occurring only in rare instances in normal closed buds (0.26%), (73).

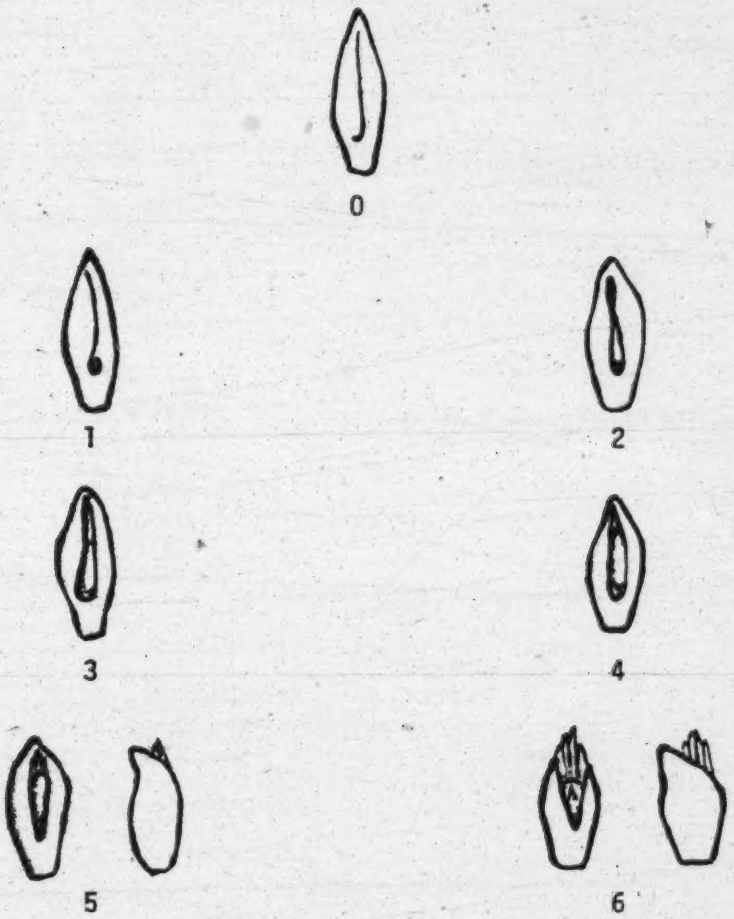


FIGURE 2: Stages in Open Bud Formation Corresponding to the Degree of Aberration of the Habit of the Buds (74).

Stage 0: normal closed bud with overlapping leaf margins,
Stage 1: bud showing an opening in the first leaf at the level of the basal conjunction of the leaf margins,
Stage 2: bud showing a chink over the total length of the bud or at its top, caused by the disappearance of the overlap of leaf margins,
Stage 3: bud with an opening at the tip,
Stage 4: bud with a wider opening than in Stage 3, through which the stamens are visible,
Stage 5: bud showing the outgrowth of stamens through the opening at the tip of the bud,
Stage 6: bud showing a degree of outgrowth of the stamens such that these organs project above the tip of the bud.

b. Flower-Bud Blasting

The term 'flower-bud blasting' is applied to the phenomenon in which flowering fails to some degree as a consequence of stagnation in the development of one or more floral organs. This disorder, in contrast to bud necrosis, manifests itself with dry necrotic symptoms. The decay and the discoloration associated with bud necrosis do not occur (76).

Flower-bud blasting can be induced by ethylene, applied at concentrations of 0.3 ppm and higher, during the storage of the bulbs. More severe symptoms are observed in bulbs treated in the later stages of storage, and at higher concentrations of ethylene (76). During the cooling period bulbs are practically resistant to ethylene; bulbs showed hardly any blasting even when exposed to ethylene concentrations of 100 ppm (76). Thus, the threshold concentration for flower-bud blasting varies with the growth stage of the flower-bud.

The effects of ethylene may be direct or delayed. In the case of the latter, storage of bulbs after ethylene treatment caused an increase in the extent and percentage of blasting (76). In the case of the former, the onset of the susceptible period is rather sudden, but this period continues up to some days before flowering (45, 78).

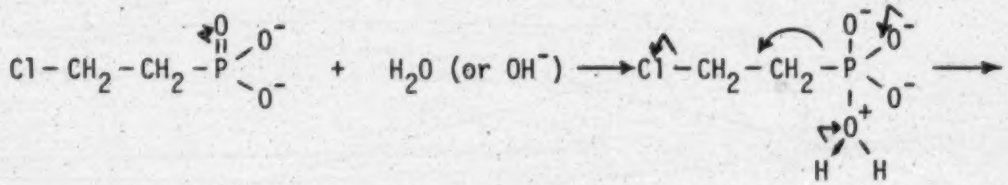
Flower-bud blasting has been interpreted as a senescence phenomenon, with ethylene as the senescence-inducing agent. Flower-bud blasting may range from mild forms to the more severe forms. In the case of the latter the bud is white and papery and the top leaf does not unfold (76). Ethylene treatment of tulips during the early forcing period (last few weeks before the expected flowering date) leads to the severe form of flower-bud blasting (direct effect). Ethylene treatment after this period leads to only very mild forms of blasting, characterized by rather flaccid tepals and 'drooping' flower heads similar to the ethylene-induced wilting

in orchid flowers (23, 31) and 'sleepiness' in carnations (103).

C. 2-Chloroethylphosphonic Acid as a Source of Ethylene

It was as early as 1946 that 2-chloroethylphosphonic acid (CEPA) was shown to give ethylene, chloride and phoshponate in alkaline solutions (49). In more recent times (107) CEPA was also shown to break down into the above components in the presence of pea epicotyl tissue.

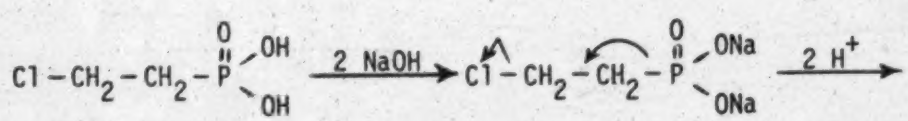
The chemical mechanism of ethylene production from CEPA was first suggested by Maynard and Swan (63). It involved the nucleophilic attack on the phosphonate dianion by a water molecule, and the concerted elimination of chlorine, leading to direct formation of phosphate and chloride as shown in Equation 1.



$$Cl^- + CH_2{=}CH_2 + H_2PO_4^- \text{ (or } HPO_4^{=})$$

EQUATION 1

Warner and Leopold (107) later postulated the following mechanism for the evolution of ethylene from CEPA (Equation 2). It involved the removal of the phosphonate dianion as a phosphonate salt, followed by dehydrohalogenation.

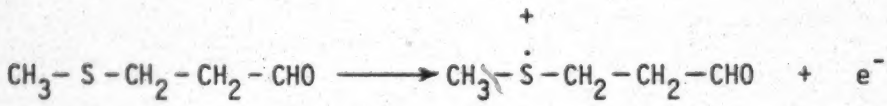


$$Na^+Cl^- + CH_2{=}CH_2 + NaHPHO_3$$

EQUATION 2

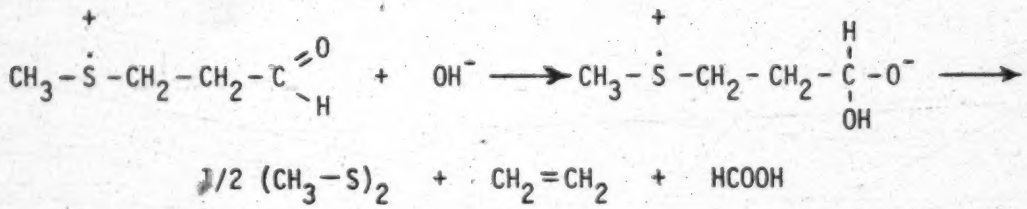
This mechanism, however, did not explain either the removal of the phosphonate group or the formation of ethylene.

Studies on the biosynthesis of ethylene (110) have shown that methional may well be related to the precursor of ethylene *in vivo*. Thus, it is quite probable that the production of ethylene from CEPA (synthetic) and methional (natural) may follow similar patterns. The decomposition of methional by the sulphite-oxygen peroxidase system (109) involved the initial extraction of an electron from the sulphur atom (Equation 3).



EQUATION 3

This was followed by a concerted nucleophilic attack of the OH^- ion or of water on the aldehyde carbon and elimination of methylmercapto radical, thus leading to the formation of ethylene, formic acid and methyldisulphide (Equation 4).



EQUATION 4

The mechanism depicted in Equation 4 is identical to that in Equation 1, for ethylene production from CEPA. Yang (111) is in favour of the mechanism of ethylene production from CEPA suggested by Maynard and Swan (63).

Whatever the mechanism of ethylene production is, the presence

of base or water is required for its evolution. In solutions more acidic than pH 3, the conversion of CEPA to ethylene was very slow, while rapid decomposition occurred at more alkaline pH levels (34). Warner and Leopold (107), in their study on the production of ethylene from CEPA, found a logarithmic type of time curve in the presence of added base; thus suggesting a second order reaction. Rate of ethylene evolution can therefore be increased by higher levels of added alkali. Their data (107) indicated no optimum pH for ethylene evolution. Rather increasing the pH enhances the rate of ethylene evolution. The minimum pH at which ethylene was released after one and one-half hours was pH 7. In buffered solutions ethylene evolution proceeds linearly with time for the first 7 hours, whereas with unbuffered solutions a logarithmic curve is obtained (107).

D. Extraction and Determination of Nucleic Acids and Protein from Plant Material

The analysis of plant nucleic acids entails great difficulties caused by the small amounts of nucleic acids relative to other compounds which interfere with the determination. In ageing plants or plant parts the amount of nucleic acids are particularly minute compared to other substances. In green members the problem is further complicated by pigments.

Kulaeva and Popova (55) studied the quantitative and qualitative aspects of several methods of extracting plant nucleic acids. Their studies revealed that plant nucleic acids and proteins may be differentially extracted by varying the perchloric acid concentration after the sodium hydroxide hydrolysis of these components. They removed the interfering substances (lipids and pigments) with an ethanol:diethyl ether (2:1) wash. Pilet and Braun (82) improved the above method with the introduction of an initial TCA:Acetone wash which extracted the lipids and

pigments prior to the sodium hydroxide hydrolysis.

DNA determination by the diphenylamine method of Burton (24) involves the reaction of diphenylamine with the sugar derived from the purine nucleotides of DNA. Interference from RNA was very slight (5 mg of commercial yeast RNA giving the same reading as 0.011 mg DNA). Interference from fructose, sucrose, starch, glycogen, glucosamine, ascorbic acid, rhamnose, ribose, acid-hydrolyzed casein and tryptophan was even less.

RNA determination involving orcinol is dependent on the pentose reaction with this reagent. The boiling time of the reaction is important because prolonged boiling increases the colour intensity and causes the precipitation of the coloured compound, even at relatively low concentrations (38). Forty minutes of boiling is sufficient to account for all the furfural formed from the pentose and pentose purine nucleosides and nucleotides; no furfural is formed from pyrimidine nucleotides.

Protein determination by the Lowry method (58) enjoys the simplicity of operation and is only less sensitive to the determination of protein by the Nessler method. However, there are two major disadvantages of the Folin reaction, namely:-

a. the amount of colour varies with the different proteins,
b. the colour is not strictly proportional to the concentration; there are two sets of curves, one below and one above 100 μg protein.

The pH of the medium is important; so is the consistency in the addition of the Phenol Reagent. When the Phenol Reagent is added to the copper treated protein, maximum colour results if the reduction occurs at about pH 10. At this pH, however, the reagent is reactive for a short period only, and it is because of this that a few seconds delay in complete mixing will lessen the amount of colour.

III. MATERIALS AND METHOD

A. Materials

Two cultivars of tulips were used in the experiments viz. cv. Darwin 33 and cv. Paul Richter. The former was obtained from a local store and the latter was obtained from a commercial supplier - Superior Bulbs (1971) Limited, Mississuaga, Ontario.

Routine chemicals of reagent grade (meeting A.C.S. specifications) were bought from Canadian Laboratory Supplier, Fisher Scientific Company and British Drug House Company. Other chemicals viz. trichloroacetic acid ('Baker Analyzed' Reagent) and orcinol ('Baker') were obtained from J.T. Baker Chemical Company. Diphenylamine (Special Indicator Grade), and acetaldehyde were obtained from Fisher Scientific Company. RNA (yeast extract) and protein (Bovine Serum Albumin) were obtained from Sigma Chemical Company, and DNA was obtained from Matheson Coleman and Bell - Manufacturing Chemists. Phenol Reagent (Folin and Ciocalteu Formula) for protein determination was obtained from Canadian Laboratory Supplier. 2-Chloroethylphosphonic acid (CEPA. 'Amchem 86-250', Ethrel*) was from Amchem Products Incorporated.

B. Methods

The centrifugations were performed on a Sorvall Superspeed Centrifuge (Type SS-1) kept in a cooler at 4 C. The ethylene determinations were made using a Varian Aerograph (Model 1740) gas chromatograph equiped with a flame ionization detector. The absorbances were measured on a Turner Spectrophotometer (Model 330).

1. Extraction of Nucleic Acids and Protein

Two methods of extraction were examined initially, phenol

* Amchem's Brand of 2-Chloroethylphosphonic acid.

extraction and trichloroacetic acid extraction; however, only the latter method of extraction was maintained because it proved to be more convenient to use as well as giving a better separation of the components.

a. Phenol Extraction (Figure 3)

The frozen sample was homogenized in a cold mortar (0 C) with cold (4 C) phosphate buffer (0.03 M potassium phosphate buffer, pH 7.0). The mixture was then transferred to a centrifuge tube and brought to a volume of 5.0 ml with the phosphate buffer. 0.5 ml of bentonite suspension (40 mg/ml) and 5.0 ml of cold water-saturated phenol were added to the suspension. The mixture was then shaken for 20 minutes and centrifuged for 15 minutes at 3500 rpm and 4 C. The upper (aqueous) layer (S1) was transferred to another centrifuge tube with a dropping pipette and stored at 4 C. 1 ml each of the phosphate buffer and water-saturated phenol were added to the bottom layer (P1) and the mixture shaken and centrifuged, as above. The upper layer (S2) was transferred to the same tube as that containing S1. This extraction was repeated again, thus giving S3, which was added to S1 and S2, the bottom layer resulting after the third extraction was discarded.

Cold (4 C) 95% ethanol was added to the accumulated aqueous fractions (S1, S2 and S3) to bring the final alcohol concentration to 65%. The mixture was shaken and then allowed to stand (20 hours at 0 C) for complete precipitation of RNA.

This mixture was then centrifuged for 5 minutes at 2000 rpm and 4 C. The supernatant obtained was discarded and the precipitate (RNA) was dissolved in 2 ml of water. The RNA was reprecipitated by the addition of 0.5 ml 10 M lithium chloride solution and the mixture centrifuged for 3 minutes at 3500 rpm and 4 C. The supernatant was again discarded and the precipitate (RNA) dissolved in water and then made to volume in a

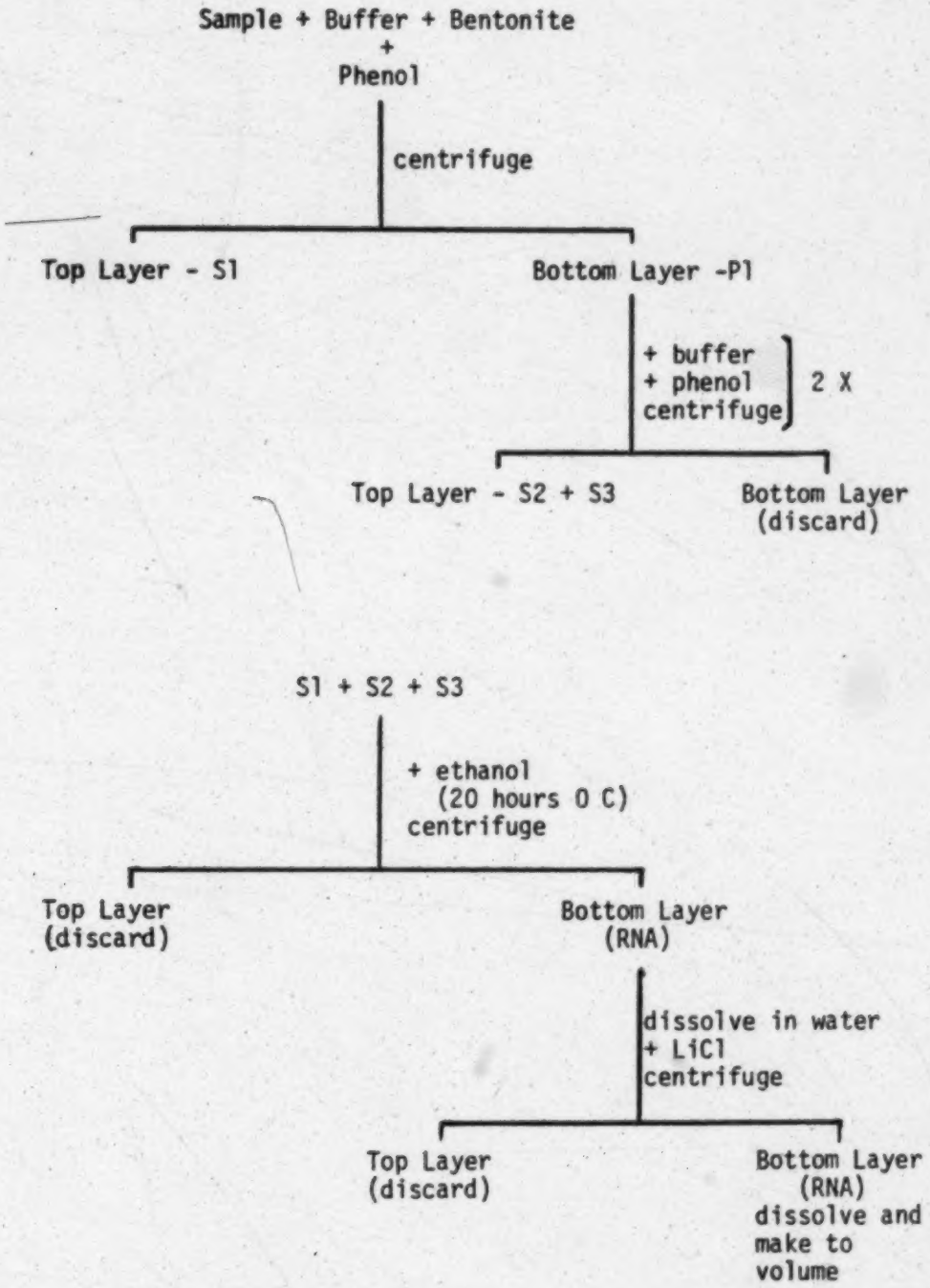


FIGURE 3: Phenol Extraction of Nucleic Acids

volumetric flask. This solution was used for RNA determination by the Orcinol method (38).

b. Trichloroacetic Acid Extraction (Figure 4)

The frozen sample was ground in a cold (0 C) mortar. After sufficient homogenization 6.0 ml of trichloroacetic acid (TCA)-acetone (5.0 g TCA in 47.5 ml water and 47.5 ml acetone) were added. After further homogenization the suspension was centrifuged for 15 minutes at 4 C and 2000 rpm.

Supernatant S1 was discarded and precipitate P1 kept for extraction. P1 was washed with a further 6.0 ml of TCA-acetone and the suspension centrifuged as above. Supernatant S2 was discarded and P2 kept for subsequent processing.

6.0 ml of 0.25 N perchloric acid (PCA) was added to precipitate P2, and after mixing the suspension was centrifuged as above. This was repeated once.

Supernatant S3 was discarded and precipitate P3 hydrolyzed for 18 hours at 37 C with 0.4 N NaOH. After the hydrolysis 1.5 N PCA was added to precipitate DNA and protein. The suspension was allowed to stand for 2 hours at 4 C to ensure complete precipitation. Subsequently the suspension was centrifuged for 15 minutes at 4 C and 2000 rpm. Supernatant S4 (RNA) thus obtained was stored in the cold (4 C), while 5.0 ml of 0.25 N PCA was added to precipitate P4. After thorough mixing the suspension was centrifuged as above. Supernatant S5 was added to S4 and the solutions brought to volume with 0.25 N PCA. This solution was used for RNA determination by the Orcinol method (38).

Precipitate P5 was hydrolyzed for 15 minutes at 70 C with 0.5 N PCA. The mixture was then allowed to cool before being centrifuged for 15 minutes at 4 C and 2000 rpm. Supernatant S6 (DNA) was stored in the

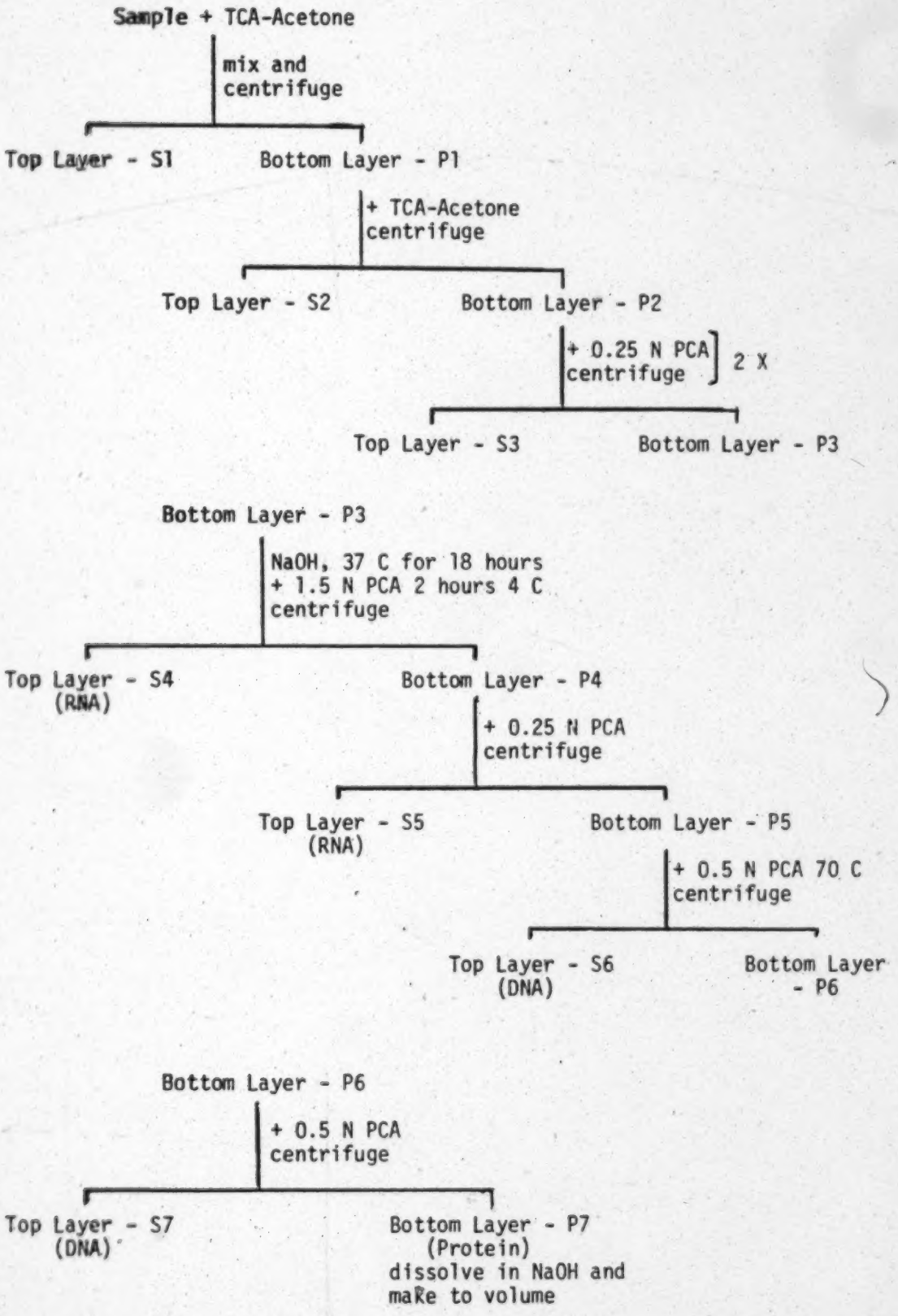

Sample + TCA-Acetone
mix and centrifuge
Top Layer - S1
Bottom Layer - P1
+ TCA-Acetone centrifuge
Top Layer - S2
Bottom Layer - P2
+ 0.25 N PCA centrifuge
2 X
Top Layer - S3
Bottom Layer - P3
Bottom Layer - P3
NaOH, 37 C for 18 hours + 1.5 N PCA 2 hours 4 C centrifuge
Top Layer - S4 (RNA)
Bottom Layer - P4
+ 0.25 N PCA centrifuge
Top Layer - S5 (RNA)
Bottom Layer - P5
+ 0.5 N PCA 70 C centrifuge
Top Layer - S6 (DNA)
Bottom Layer - P6
Bottom Layer - P6
+ 0.5 N PCA centrifuge
Top Layer - S7 (DNA)
Bottom Layer - P7 (Protein) dissolve in NaOH and make to volume

FIGURE 4: Trichloroacetic Acid Extraction of Nucleic Acids

cold (4 C) while precipitate P6 was resuspended in 0.5 N PCA and centrifuged as above. Supernatant S7 was added to S6 and the solutions made to volume with 0.5 N PCA. This was the DNA solution used in the determination of DNA by the diphenylamine reagent (24).

Precipitate P8 (protein) was dissolved in 10 ml NaOH. This formed the protein solution used for the determination of protein in the sample by the Lowry method (58).

2. RNA Determination

The RNA determination was performed according to the method of Geriotti (38).

Test tubes containing 0, 0.5, 1.0, 1.5, 2.0 and 3.0 ml of the standard RNA solution (50 mg/l) in 0.25 N PCA and suitable quantities of the RNA solution from the extract were brought to 3.0 ml with 0.25 N PCA.

3.0 ml of the orcinol solution (1% recrystallized orcinol in HCl with 0.1 % $FeCl_3$) was added to each tube. The resulting mixture was then mixed for 30 seconds in a Vortex mixer (Speed #2).

The tubes were loosely stoppered and then placed in a boiling water bath for 40 minutes. After the 40 minutes were up, the tubes were cooled immediately under running water. The absorbances were read at 675 nm. The absorbances of the standard were plotted against the RNA concentrations of these solutions and the RNA in the solution obtained from the extract read off from the standard curve.

3. DNA Determination

The standard DNA solution was prepared by hydrolyzing the DNA for 15 minutes, at 70 C, in 0.5 N PCA (50 µg/ml).

Test tubes containing 0, 0.25, 0.50, 0.75, 1.00, 1.50 and 2.00 ml of the standard DNA solution, as well as test tubes containing

suitable quantities of the DNA solution of the extract was brought to 2 ml with 0.5 N PCA.

4 ml of freshly prepared diphenylamine-acetaldehyde solution [1.5 g diphenylamine dissolved in 100 ml glacial acetic acid and 1.5 ml concentrated sulphuric acid; immediately before use 0.5 ml of acetaldehyde solution (2 ml acetaldehyde puriss in 100 ml water) was added to the diphenylamine reagent] was added to each of the test tubes. The test tubes were then loosely stoppered and shaken for 30 seconds in a Vortex mixer. The tubes were then placed in the dark. Colour development at room temperature reached a maximum and was stable after a 6-hour period.

The absorbances were read at 600 nm. Absorbances were plotted against the DNA concentrations of the standard and the DNA values of the extract read off from this curve.

4. Protein Determination

The solutions used in the determination according to the method of Lowry et al (58) were as follows:-

Protein Standard - 100 μg/ml bovine serum albumin in 1.0 N NaOH,

Solution A - 2% Na_2CO_3,

Solution B - 1% $CuSO_4$ solution mixed with an equal volume of a 2% sodium potassium tartarate solution, immediately before use,

Solution C - 50 ml of Solution A and 1.0 ml Solution B mixed immediately before use,

Solution D - 1.0 ml Phenol (Folin-Ciocalteau) Reagent added to 1.36 ml water.

Test tubes containing 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the standard protein solution and suitable quantities of the protein solution of the extract were brought to 1.0 ml with 1.0 N NaOH solution.

4.0 ml of Solution C was added to each of the test tubes. The test tubes were shaken and then allowed to stand for 10 minutes at room temperature.

5.0 ml of Solution D was then added to each of the test tubes with instantaneous and vigorous mixing. The absorbance readings were taken after 30 minutes, and the absorbances plotted against the protein concentrations. The protein concentration of the extract was then determined from this standard curve.

5. Ethylene Determination

Ethylene was estimated by gas chromatography using a 5 feet (1.5 m) x I/8 inch (3.1 mm) stainless steel column, packed with activated alumina (60-80 mesh). The gas was injected directly into the column through a septum, at the injection port, by means of a syringe. The temperature settings were as follows:-

Column - 50 C, injection port - 125 C and detector - 200 C.

The carrier gas was nitrogen with a flow rate of 24 ml per minute.

The peak heights in the chromatogram were plotted against known amounts of standard ethylene in ppm. From these calibration curves the amount of ethylene in the unknown sample was determined from the peak height of that sample. The instrumental error was kept to a minimum by maintaining the same conditions in all runs.

6. Production of Ethylene from 2-Chloroethylphosphonic Acid

The conversion of 2-chloroethylphosphonic acid (CEPA) to ethylene in the presence of sodium hydroxide is relatively high; a 98% efficiency is obtained after 48 hours (107).

Calculations:

Average atmospheric pressure in Edmonton at 25 C = 934.3 millibars

= 700.78 mm Hg.

Thus pressure at 18 C = [(700.78 x 291)/298] = 684.32 mm Hg.

Volume occupied by one mole of a gas at S.T.P. is 22.4 liters.

Thus volume occupied by one mole of ethylene at 18 C and 684.32 mm Hg

$$= 22.4 \times \frac{760}{684.32} \times \frac{291}{273} = 26.5175 \text{ liters.}$$

Capacity of the plexiglass cabinet = 486 liters.

Thus for a 10 ppm ethylene concentration, the gas should occupy

$$4860 \times 10^{-6} \text{ liters.}$$

Under experimental conditions this would require

$$\frac{4860 \times 10^{-6}}{26.5175} = 183.3 \times 10^{-6} \text{ moles of ethylene.}$$

Concentration of CEPA solution (Amchem 68-250, Ethrel*) was

4 lb/gal or 0.4793 g/ml.

Molecular Weight of CEPA is 237.5

Thus Molarity of CEPA solution = $\frac{0.4793}{237.5} \times 1000 = 2.018$ M

Assuming that the conversion of CEPA to ethylene is 98% (107),

Then 1 liter of the CEPA solution would give 2.018 x 0.98 = 1.977 moles of ethylene.

Thus for a 10 ppm ethylene concentration, in the cabinet, under these conditions the volume of CEPA required is

$$\frac{183.3}{1.977} \times 10^{-6} \text{ liters} = 92.7 \text{ microliters.}$$

The determination of the ethylene concentration in the cabinet after 48 hours by gas chromatography revealed a concentration of 8.83 ppm. The volume of the CEPA solution was then adjusted to 104.96 microliters to give a 10 ppm ethylene concentration. This value was confirmed by gas chromatography.

The volume of the sodium hydroxide used was 5 ml of a 5.0 N NaOH solution. At this volume and concentration of sodium hydroxide the release of ethylene from CEPA was almost instantaneous.

The lower yield of ethylene obtained to that predicted from the calculations may have been due to:-

a. A lower efficiency in the conversion of CEPA to ethylene,

b. The concentration of the CEPA given was not accurate.

The required ethylene concentrations for the experiments were thus obtained using the adjusted volume of the CEPA solution, i.e. 104.96 microliters of the CEPA solution for a 10 ppm ethylene concentration within a 486 liter capacity. When the 128 fluid oz jars were used the volume of the CEPA was adjusted correspondingly.

7. Treatment of the Bulbs (Figure 5)

Bulbs (cv. Paul Richter) were checked for the stage of flower development after they were received from the commercial supplier (October 13). This investigation was carried out according to the method described in 'The Ball Red Book' (9). All the bulbs examined at random (20 bulbs) had reached the G-stage (Figure 1). These bulbs were divided into groups, Group A and Group B. Group A was placed directly into cold storage (10 C), while Group B was planted out in the cold frame in a soil/peat mixture (October 15). These bulbs were planted 12 cm apart and 20 cm deep.

a. Bulbs (cv. Paul Richter) from the Commercial Supplier

These were the bulbs in Group A which were placed into cold storage upon arrival. The effect of ethylene on these bulbs during the cooling and forcing periods was studied.

i. Effect of Ethylene During the Cooling Period (10 C)

10 bulbs (cv. Paul Richter, G-stage) were used for each treatment. These bulbs were placed in sealed glass jars (128 fluid oz) and the ethylene atmosphere generated by the action of sodium hydroxide on CEPA. The ethylene concentrations were confirmed by gas chromatography.

The bulbs (cv. Paul Richter, G-stage) were exposed to 0,

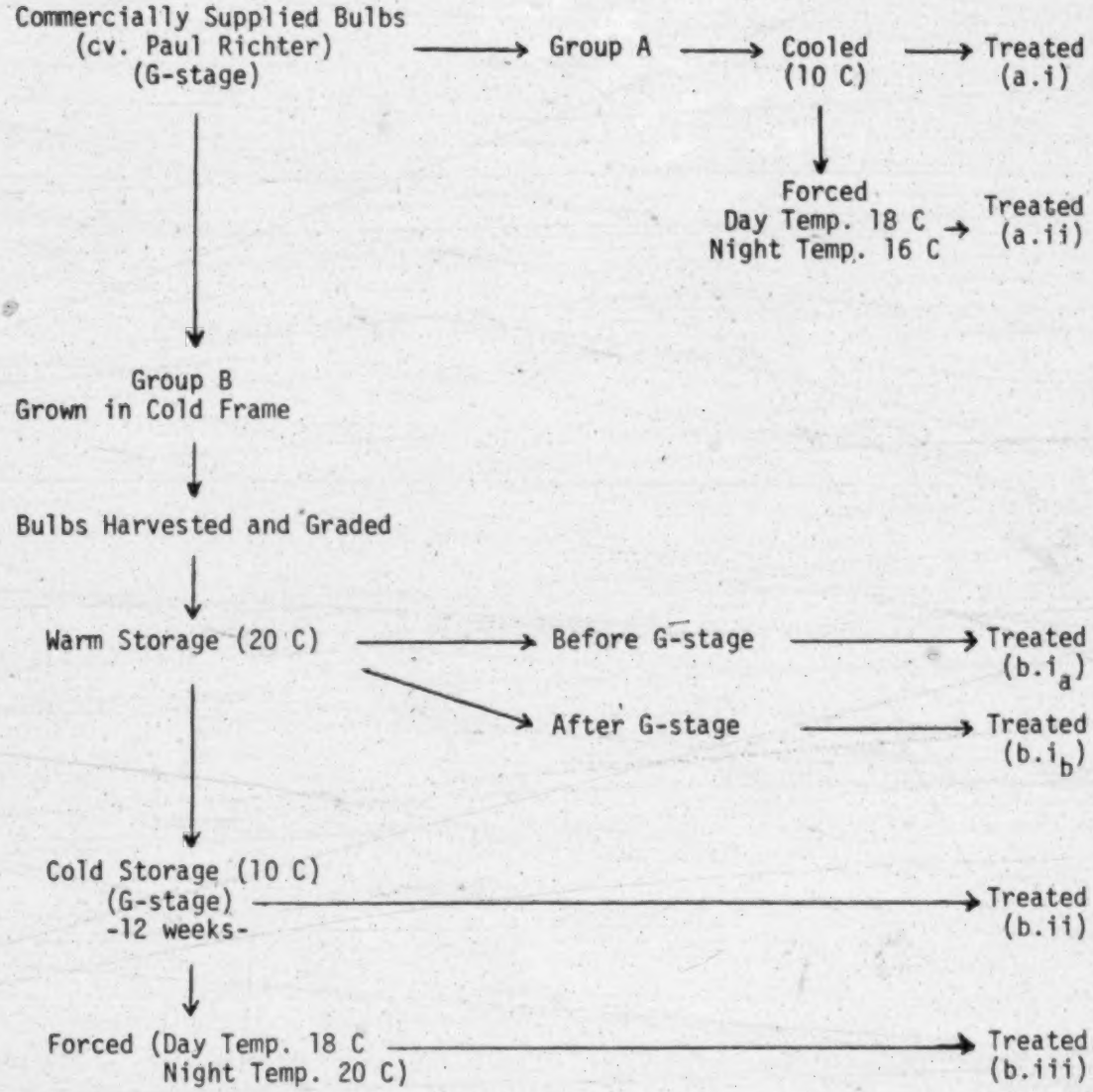


FIGURE 5: Treatment of the Bulbs

5 and 10 ppm ethylene for 2- and 4-week periods. Immediately after the exposures the jars were opened and 2 bulbs from each treatment were removed for the determination of RNA and protein. The flower-buds were dissected out and frozen immediately. They were kept in the freezer up to the extraction time. At one week intervals two bulbs from each treatment were taken for analyses.

ii. Effect of Ethylene During Forcing (Day Temperature 18 C, Night Temperature 16 C)

Bulbs (cv. Paul Richter, G-stage) were planted in a 3:2:1 soil mixture (loam:peat:sand) and kept in cold storage (10 C) for 12 weeks (cooling period) before being forced (day temperature 18 C, night temperature 16 C).

The plants were placed in gas tight plexiglass cabinets (90 cm x 90 cm x 60 cm) during the treatment. The plants were exposed to 0, 5 and 10 ppm ethylene concentrations for a one week period. As in the above experiment, the ethylene atmosphere was generated from CEPA and sodium hydroxide. The ethylene concentration within these cabinets were checked after 48 hours by gas chromatography.

b. Bulbs (cv. Paul Richter) from the Cold Frame

The first generation bulbs from the bulbs (cv. Paul Richter) of Group B, grown out in the cold frame, were harvested in mid-July because of adverse weather conditions. The bulbs were harvested by hand and the foliage allowed to dry before being removed. Only bulbs from plants that gave normal flower parts (tepals - 6, stamens - 6, carpels - 3) were collected. The bulbs were then checked for blemishes, only healthy looking bulbs were collected, the others were discarded. These bulbs were placed in a single layer in perforated plastic trays and stored at 20 C.

The stage of flower development of the bulbs was checked

initially at 2 week intervals, and later increased to weekly intervals once the foliage initials had been laid down.

i. Effect of Ethylene During the Postharvest Maturation Period (20 C)

The effect of ethylene during this period may be divided into two phases:-

a. the period prior to complete flower formation (before G-stage),

b. the period after complete flower formation (G-stage).

i_a. Prior to complete Flower Formation (Before G-stage)

The bulbs used in this experiment contained buds in which only the foliage leaf initials had been laid down (Figure 1 - Stage I). The bulbs (cv. Paul Richter and Darwin 33), in sealed glass jars (128 fluid oz), were exposed to 0 - 10 ppm ethylene for one week periods. After the ethylene exposure the jars were opened, and when the buds of these bulbs had reached the G-stage (Figure 1) the bulbs were planted in the 3:2:1 soil mixture (loam:peat:sand). They were then placed in a 10 C room for the 12 week cooling period, before being forced under the usual conditions (day temperature 18 C, night temperature 16 C). Throughout the cooling and forcing periods the soil was kept moist, but not water-logged.

i_b. After Flower Formation (G-stage)

Bulbs (cv. Paul Richter) in which flower formation was complete (G-stage, Figure 1) were exposed to 0 - 5 ppm ethylene for a 4-day period at 20 C. This treatment was performed in the dark and in the 128 fluid oz glass jars. The ethylene atmosphere was generated by CEPA and sodium hydroxide and the concentration confirmed by gas chromatography.

Immediately after the 4-day treatment the jars were opened and 2 bulbs from each treatment were taken for analyses. Subsequent analyses were carried out 1 and 2 weeks after the ethylene exposure.

ii. Effect of Ethylene During the Cooling Period (10 C)

The first generation crop of bulbs (cv. Paul Richter) from 'normal' plants grown out in the cold frame were placed in cold storage (10 C) when the embryonic flower had reached the G-stage (Figure 1). These bulbs were then treated during this period. Bulbs (cv. Paul Richter) were exposed to 0 and 0.5 ppm ethylene concentrations, the ethylene being generated as in the other experiments by the reaction of CEPA and sodium hydroxide. The length of the ethylene exposure varied from one to five weeks.

Bulbs were removed immediately after the 1-, 2-, 3-, 4- and 5-week exposure periods and the flower-buds analyzed for the nucleic acids and protein. Bulbs from each treatment were also analyzed one week after the ethylene removal.

iii. Effect of Ethylene During Forcing (Day Temperature 18 C, Night Temperature 16 C)

Bulbs (cv. Paul Richter) of the first generation crop, were planted in a 3:2:1 soil mixture (loam:peat:sand) after the bulbs had attained the G-stage (Figure 1). The potted bulbs were kept moist during the 12-week cooling period (10 C). The plants were then forced at a day temperature of 18 C and a night temperature of 16 C. The flower-buds were still within the last foliage leaves when the plants were forced.

These plants were placed in the 90 cm x 90 cm x 60 cm gas tight plexiglass cabinets and treated with 0, 0.1, 0.3 and 0.5 ppm ethylene concentrations for periods of 2, 7 and 10 days. The ethylene atmosphere was supplied by the reaction of CEPA and sodium hydroxide. The flower-buds from each of the treatments were analyzed immediately after the ethylene exposure for the nucleic acids and proteins.

IV. RESULTS AND DISCUSSION

A. Extraction Procedure

The basic aim of the extraction procedure was to extract the nucleic acids and protein of the plant material quantitatively. Two methods of extraction were compared (phenol and trichloroacetic acid extractions) to determine their efficiency.

1. Phenol Extraction

In the phenol extraction technique, without the addition of sodium dodecyl sulphate to the extraction media, both DNA and protein are left in the bottom (phenol) layer, while only RNA goes into the top (aqueous) layer. This separation, however, is not complete; some of the more firmly bound nucleic acids will remain bound to the protein even after the phenol treatment and hence, will enter the bottom instead of the top layer. In addition, the nucleases are not completely inactivated by phenol; thus some degradation of the nucleic acids will occur, resulting in lower yields.

Tests on the efficiency of the extraction of RNA by phenol revealed that a minimum of 3 extractions were necessary to obtain a 99% efficiency (Table 1). The number of extractions required may have been caused by the difficulty encountered in removing the top (RNA) layer completely.

Recovery of RNA from the aqueous phase was achieved by precipitation with ethanol. The precipitation of RNA varied with the ethanol concentration (Table 2). Thus the ethanol concentration of the media had to be adjusted to better than 60% for a 98% precipitation of the nucleic acid. These results were in agreement with those obtained by Harrison (41).

Generally, the phenol extraction technique gave lower yields

TABLE 1: Efficiency of the Phenol Extraction

Extraction Number	RNA Recovered (%)	
1	92.98	
2	4.17	98.90
3	1.75	
4	0.58	
5	0.52	

A known amount of RNA was mixed with the extraction solution (phosphate buffer, bentonite solution, water saturated phenol) and the mixture centrifuged. The top aqueous layer was then removed and the RNA extracted determined. The bottom layer was reextracted with phosphate buffer and phenol and after centrifugation the RNA extracted determined. This procedure was repeated until all (or most) of the RNA added had been accounted for.

TABLE 2: Effect of Ethanol Concentration on Precipitation of RNA

Ethanol Concentration (%)	RNA Precipitated (%)
5	61.1
10	62.0
20	68.2
30	70.1
35	70.4
40	78.4
45	92.3
50	96.8
55	97.2
60	98.4
70	99.2

The required ethanol concentrations were obtained by the addition of appropriate amounts of cold (4 C) 95 % ethanol to the aqueous fractions obtained after phenol extraction. The mixture was shaken and allowed to stand for 18 hours at 0 C before being centrifuged for 5 minutes at 2000 rpm and 4 C. The RNA precipitated was determined by the orcinol method.

of nucleic acids. This may have been caused by incomplete extraction and/or incomplete precipitation. In addition, the amount of nucleic acid extracted was not quantitative; nucleic acids which were bound firmly to proteins were not extracted and degradation of the nucleic acids is possible as the nucleases are not completely inactivated by phenol. Thus, this method of extraction was considered unsuitable for this study.

2. Trichloroacetic Acid Extraction

The trichloroacetic acid extraction technique gave easily soluble degradation products. It also provided a simple means of separating the nucleic acids and proteins into separate components which could then be determined spectrophotometerically.

The method involved an initial precipitation of the nucleic acids and proteins. The pigments and lipids were removed at the same time by the introduction of acetone into the extraction media (82). This removed the necessity of a separate step, for the removal of these components, which was essential when only trichloroacetic acid was employed (55, 82).

Total hydrolysis of DNA, RNA and protein was achieved after an 18 hour incubation with sodium hydroxide at 37 C. The subsequent separation of the components into DNA, RNA and protein was complete. No detectable quantities of DNA appeared in either of the RNA or protein fractions.

B. DNA, RNA and Protein Determinations

DNA, RNA and protein were determined spectrophotometerically by methods devised by Burton (24), Gerrotti (38) and Lowry et al (58) respectively.

1. DNA Determination

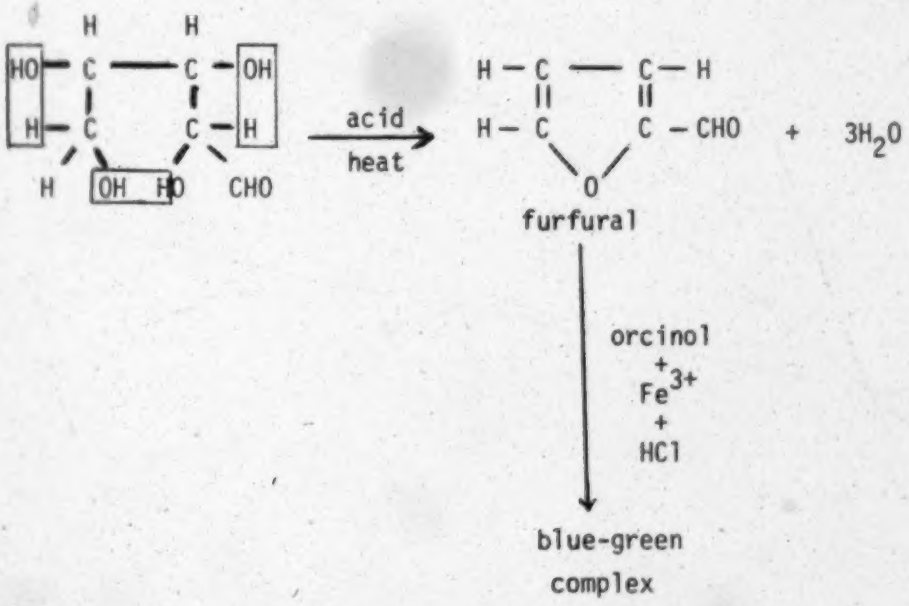
The method of DNA determination by Burton (24) involved the

reaction of the diphenylamine-acetaldehyde reagent with the sugar moieties derived from the purine nucleotides of DNA. This method is specific for DNA, interference from other substances is insignificant (24).

The DNA solutions could be incubated with the diphenylamine-acetaldehyde reagent for 6 hours at room temperature or overnight in the refrigerator (82). It was found, however, that in the latter instance the correlation between DNA concentrations and absorbances was unsatisfactory, while in the former a favourable correlation was obtained.

2. RNA Determination

The method of RNA determination by Gerrotti (38) is based on the formation of a blue-green complex between furfural and orcinol in the presence of an oxidizing agent. Equation 5 illustrates the mechanism of action of the reaction, it also shows that the reaction is specific for the pentoses (ribose of RNA, but not the deoxyribose of DNA).



EQUATION 5

Prolonged boiling, however, causes the precipitation of the coloured complex (38). The boiling time was thus standardized to 40 minutes.

hence preventing the precipitation as well as providing a valid means for comparison.

3. Protein Determination

The Lowry method (58) of protein determination had to be standardized before suitable correlation was obtained between protein concentrations and absorbances. Protein concentrations in the reaction mixtures were kept below 100 µg because above this concentration a different curve was obtained. The technique involved in the addition of the Phenol Reagent to the copper treated protein has a pronounced effect on subsequent colour development (58). To ensure maximum colour formation, the Phenol Reagent was added to the reaction mixture while the tube was agitated on a Vortex mixer (Speed #2). Under these conditions the correlation between protein concentrations and absorbances was favourable.

C. Effect of Ethylene During Different Growth Stages of the Tulip

Tulip bulbs at different stages of growth require different environmental conditions for favourable development. Hence the effect of ethylene under these conditions were examined.

1. Effect of Ethylene During the Postharvest Maturation Period (20 C)

During the postharvest maturation period the bulbs may be in different stages of growth. Bulbs immediately after lifting may not have attained complete embryonic flower formation (prior to G-stage, Figure 1), whereas bulbs maintained for some time after harvest may have their flower completely formed (G-stage). The effect of ethylene during this period was subdivided into two groups, that period:-

a. prior to complete flower formation (before G-stage),

b. after complete flower formation (G-stage).

a. Prior to Complete Flower Formation (Before G-stage)

Bulbs (cv. Darwin 33 and Paul Richter) in which the foliage initials had been laid down, but, before flower formation was complete (before G-stage) were used in this experiment. The dry, brown tunic of these bulbs was removed prior to the ethylene exposure to facilitate ethylene penetration and observation of gum blisters.

Bulbs of cultivar Darwin 33 responded to the ethylene treatment by the formation of gum blisters, while those of the Paul Richter did not, even at ethylene concentrations of 100 ppm. This may have been due to varietal differences between the bulbs (52). The blisters formed were localized on the outer surface of the outermost fleshy scale leaf of the bulb.

In the 1-week exposures of the Darwin 33 bulbs to low ethylene concentrations (less than 1 ppm) the blisters formed were small and did not burst. However, at the higher ethylene concentrations the blisters burst and gave rise to the exudation of a colourless liquid. Eventually this liquid turned viscous and yellow, and finally solified to a hard brown mass.

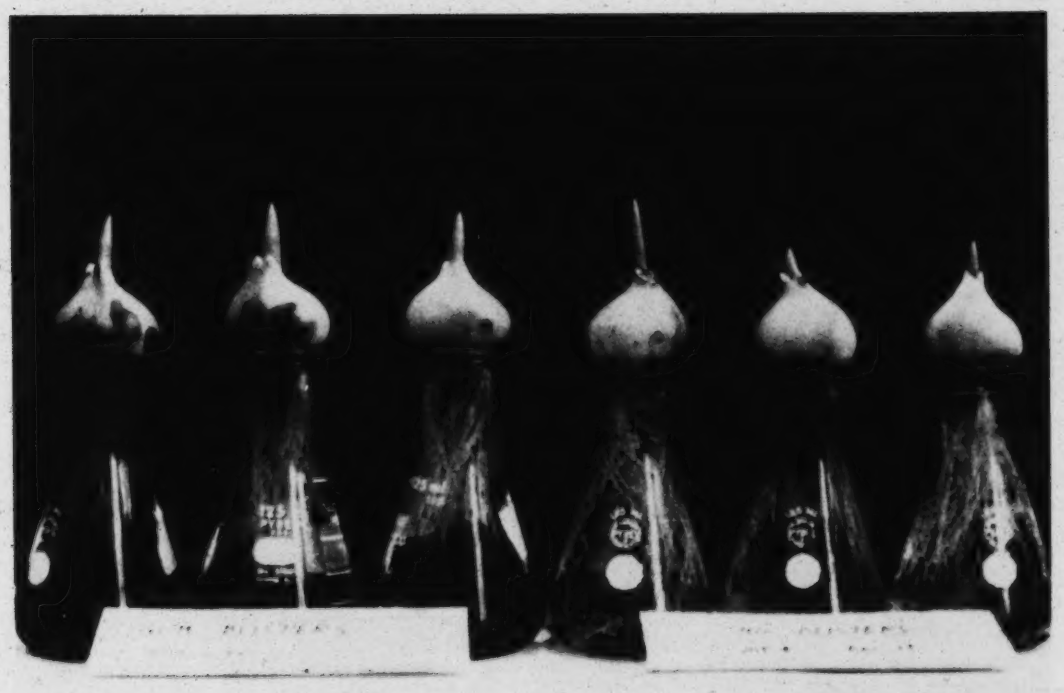
The susceptible phase of the bulbs (cv. Darwin 33) decreased with time. Thus after a storage period of 16 weeks or more, exposure of these bulbs to 100 ppm ethylene did not give rise to gum blisters.

Bulbs with gum blisters gave rise to retarded root growth (Figure 6). This substantiates the retarding effect of ethylene on root growth of tulips (76, 78).

Although the bulbs of cultivar Paul Richter did not give rise to gum blisters when treated with ethylene during the postharvest maturation period, ethylene did have an effect on the bulbs. The number of flower-parts were affected. Unlike the effect of ethylene on the flowering of cucumber (89) and pineapple (18), the gas did not have a fixed pattern

FIGURE 6: Effect of Ethylene on Gum Blister Formation and Subsequent Root Growth of Tulips (cv. Darwin 33)

The bulbs were treated with 1 ppm ethylene after lifting. The treatment lasted for one week. After the bulbs achieved the G-stage the bulbs were placed over water in flasks and given the cold treatment (10 C).
The ethylene treated bulbs show gum blisters and the subsequent root growth of the treated bulbs was retarded.



Ethylene Effects:- Gum Blisters and Root Growth

in its effect on the tulip. In certain cases the number of stamens and tepals were increased, while in others they were decreased. This random alteration in the number of floral parts could have resulted from the upset in the metabolism of the bud, caused by ethylene, while the flower initials were being laid down. Resende (88) cited references to show that flower development is carried out phylogenically by additive genes controlling native growth regulation $\frac{\Sigma FFF}{\Sigma MMM} = \frac{\text{growth promoter}}{\text{growth inhibitor}}$. Ethylene in low concentrations acts as a growth promoter, while in high concentrations it is a growth inhibitor (48). Thus, ethylene may affect the sex of the flower in either direction depending on its concentration and the sensitivity of the plant to ethylene.

In contrast to the above, ethylene treatments during the forcing period do not give alterations in the number of floral parts, although it may cause reduction in growth and increased incidence of flower-bud blasting (Figure 7.1). Besides the effect on flower structure, ethylene treatment during the postharvest maturation period while the foliage leaf initials were being laid down (Figure 1), could give rise to abnormalities in the leaf. The last leaf in some of the bulbs (cv. Paul Richter) treated during this period had a double apex and in others this leaf was petalloid (Figure 7.2). This alteration in the structure of the leaf may have been caused by ethylene affecting the metabolism of the bulb during foliage leaf differentiation. Here again Resende (88) has stated that the ratio of growth promoter to that of the growth inhibitor can influence the vegetative/reproductive phase of the plant.

b. <u>After Complete Flower Formation (G-stage)</u>

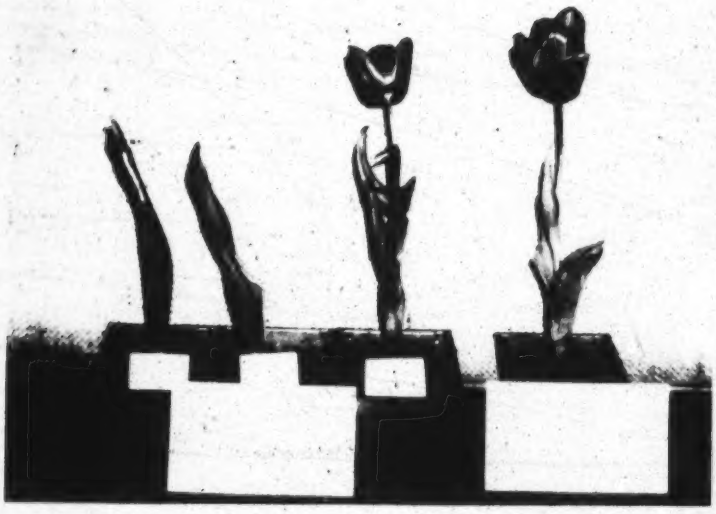
Bulbs (cv. Paul Richter), in this experiment, were the first generation crop of bulbs obtained from the commercially supplied Paul Richter bulbs (Group B) which were grown out in the cold frame (Materials

FIGURE 7.1: Effect of Ethylene During the Forcing Period

Tulip plants (cv. Paul Richter) were treated with 0.1, 0.3 and 0.5 ppm ethylene for a period of 10 days. Severe flower-bud blasting occurred with the 0.3 and 0.5 ppm ethylene concentrations, however, at the 0.1 ppm ethylene level only slight growth retardation occurred.

FIGURE 7.2: Effect of Ethylene During the Postharvest Maturation Period

Bulbs (cv. Paul Richter, Stage I) were exposed to varying concentrations of ethylene during the postharvest maturation period.
'Petalloid' leaf was observed with ethylene treatments of 3 ppm (or higher).



1.
Growth Retardation Caused by Ethylene

2.
Petalloid Leaf

and Methods). The bulbs were graded before use. Only bulbs (11-12 cm) from plants which had normal flowers and were free of blemishes were used.

The bulbs were analyzed for DNA, RNA and protein immediately after the 4-day ethylene treatment. The results are given in Table 3 and represented graphically in Figure 8. The DNA, RNA and protein levels in these bulbs one week and two weeks after the ethylene treatment are given in Tables 4 and 5, and illustrated graphically in Figures 9 and 10, respectively.

Both DNA and protein concentrations in the buds immediately after the ethylene exposure increased linearly with increasing ethylene concentrations used (Figure 8). The RNA concentration, however, reached a maximum at the 2 ppm ethylene concentration and was maintained at this level even when concentrations of the gas were increased to 5 ppm (Figure 8). These results indicate that DNA as well as the RNA and protein content of the bud increased under these conditions. Compared to DNA and protein content, the RNA content levelled off (Figure 8). This, however, does not exclude the possibility that both DNA and protein content could have attained a maximum at higher levels of ethylene concentrations.

The DNA content of the buds, one week after the 4-day ethylene exposure showed higher levels with increasing ethylene concentrations (Figure 9). Nevertheless, the difference between the DNA content of the 5 ppm treatment and control, one week after the ethylene exposure (2.37 mg/g F.W.), was lower than that immediately after the exposure (2.78 mg/g F.W.). This showed that one week after the ethylene exposure the DNA content in buds, receiving higher ethylene concentrations, had begun to decline. The same held true for the RNA and protein content, the levels declining at ethylene concentrations of 2 ppm of greater (Figure 9).

Two weeks after the 4-day ethylene exposure, the DNA, RNA and

TABLE 3: Effect of Ethylene During the Postharvest Maturation Period (20 C)

	Ethylene Concentration (ppm)					
	0	1	2	3	4	5
DNA (mg/g F.W.)	1.25	2.30	2.67	3.04	3.50	4.03
RNA (mg/g F.W.)	11.37	15.33	16.60	16.83	16.92	17.11
Protein (mg/g F.W.)	4.63	6.88	7.85	8.83	9.12	10.27
DNA/RNA	0.11	0.15	0.16	0.18	0.21	0.24
DNA/Protein	0.27	0.33	0.34	0.34	0.38	0.39
RNA/Protein	2.46	2.33	2.11	1.91	1.86	1.67

Tulip bulbs (cv. Paul Richter, G-stage) in sealed jars and in the dark, were exposed to various concentrations of ethylene for a period of 4 days. The buds from these bulbs were analyzed for DNA, RNA and protein immediately after the ethylene exposure. The TCA-Acetone method of extraction was employed.

FIGURE 8: Effect of Ethylene During the Postharvest Maturation Period

Tulip bulbs (cv. Paul Richter, G-stage) were treated with varying ethylene concentrations for a 4-day period. The figure gives the effect of ethylene concentration on the DNA, RNA and protein levels of the bud immediately after the ethylene treatment.

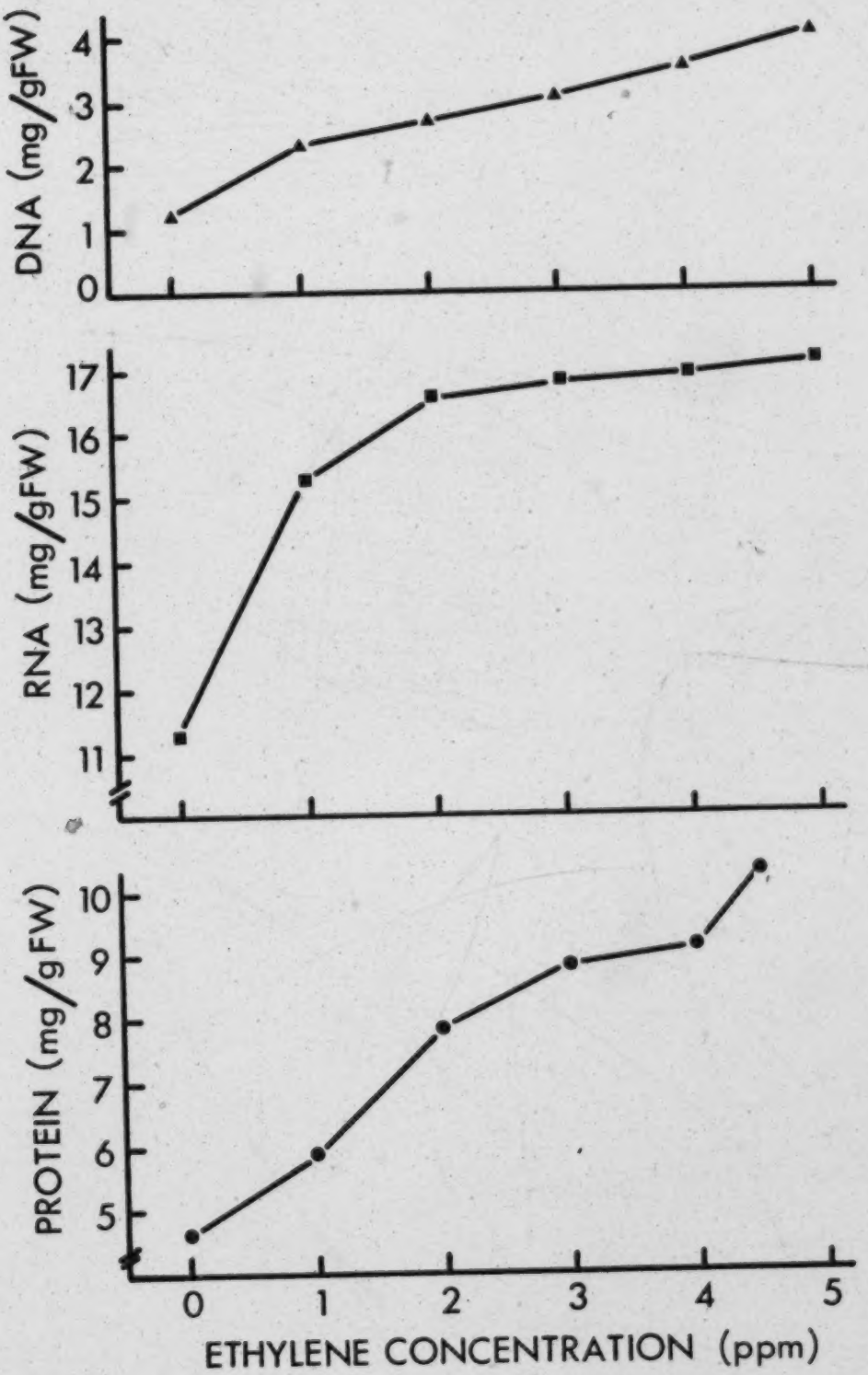

DNA (mg/gFW)
0
1
2
3
4
RNA (mg/gFW)
11
12
13
14
15
16
17
PROTEIN (mg/gFW)
5
6
7
8
9
10
0
1
2
3
4
5
ETHYLENE CONCENTRATION (ppm)

TABLE 4: Effect of Ethylene During the Postharvest Maturation Period (20 C)

	Ethylene Concentration (ppm)					
	0	1	2	3	4*	5*
DNA (mg/g F.W.)	2.87	4.25	4.40	4.55	4.75	5.24
RNA (mg/g F.W.)	15.56	17.65	20.09	18.80	19.27	17.66
Protein (mg/g F.W.)	6.81	10.18	10.65	10.83	10.25	9.12
DNA/RNA	0.18	0.24	0.22	0.24	0.25	0.30
DNA/Protein	0.42	0.42	0.41	0.41	0.46	0.57
RNA/Protein	2.28	1.73	1.89	1.74	1.88	1.94

Tulip bulbs (cv. Paul Richter, G-stage) in sealed jars and in the dark, were exposed to various concentrations of ethylene for a period of 4 days. The jars were then opened and the buds from these bulbs were analyzed for DNA, RNA and protein one week after the ethylene exposure. The TCA-Acetone method of extraction was employed.

* Stamens longer than tepals.

TABLE 5: Effect of Ethylene During the Postharvest Maturation Period (20 C)

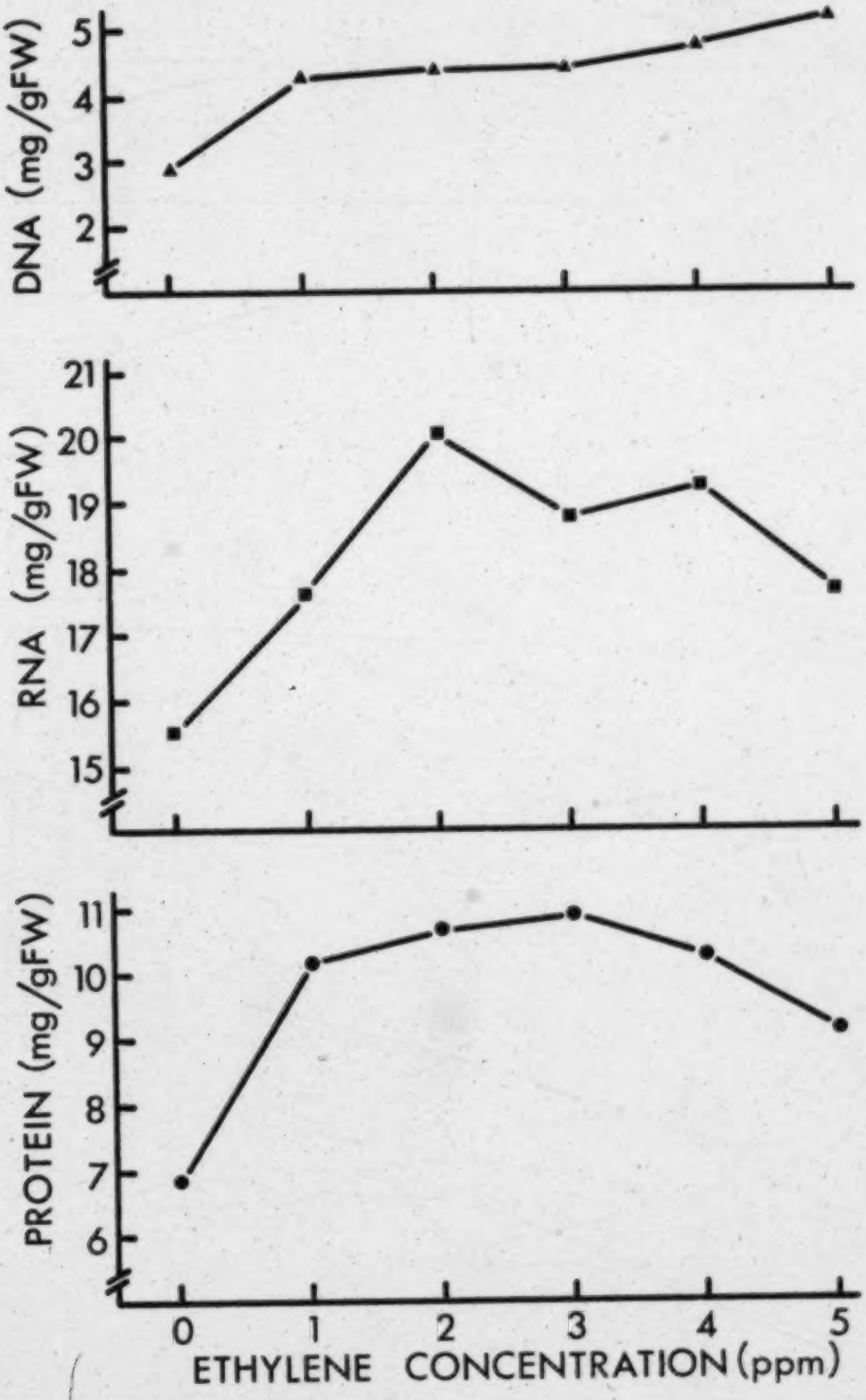
	Ethylene Concentration (ppm)					
	0	1	2*	3*	4*	5*
DNA (mg/g F.W.)	4.43	5.83	6.32	5.97	5.57	5.48
RNA (mg/g F.W.)	20.45	18.75	18.41	17.95	17.92	17.89
Protein (mg/g F.W.)	7.85	9.40	8.82	8.97	8.93	8.27
DNA/RNA	0.22	0.31	0.34	0.33	0.31	0.31
DNA/Protein	0.56	0.62	0.72	0.67	0.62	0.67
RNA/Protein	2.46	2.00	2.09	2.00	2.01	2.16

Tulip bulbs (cv. Paul Richter, G-stage), in sealed jars and in the dark, were exposed to various concentrations of ethylene for a period of 4 days. The jars were then opened and the buds from these bulbs were analyzed for DNA, RNA and protein two weeks after the ethylene exposure. The TCA-Acetone method of extraction was employed.

* Stamens longer than tepals.

FIGURE 9: Effect of Ethylene During the Postharvest Maturation Period

Tulip bulbs (cv. Paul Richter, G-stage) were treated with varying ethylene concentrations for a 4-day period. The figure gives the effect of ethylene concentration on the DNA, RNA and protein levels of the bud one week after the ethylene treatment.


DNA (mg/gFW)
5
4
3
2
RNA (mg/gFW)
21
20
19
18
17
16
15
PROTEIN (mg/gFW)
11
10
9
8
7
6
0
1
2
3
4
5
ETHYLENE CONCENTRATION (ppm)

protein content of the buds gave lower values at the higher ethylene concentrations. However, the DNA and protein content in the buds of treated bulbs were at higher levels than those in the control. This did not extend to the RNA content. The RNA levels in treated buds were lower than those in the control (Figure 10)

The alteration in the DNA content of treated bulbs supports the hypothesis that ethylene may act at the site of DNA synthesis. If, it is assumed that protein synthesis follows the following sequence (Figure 11), the increase in DNA could account for the increase noted in both the RNA and protein.

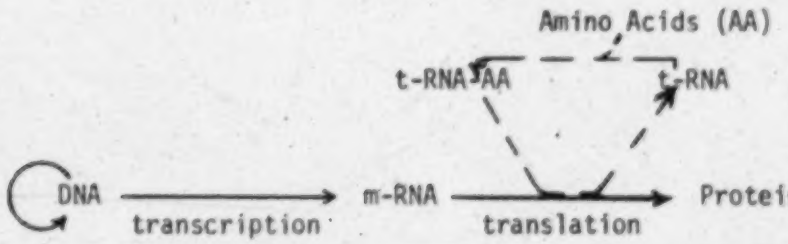
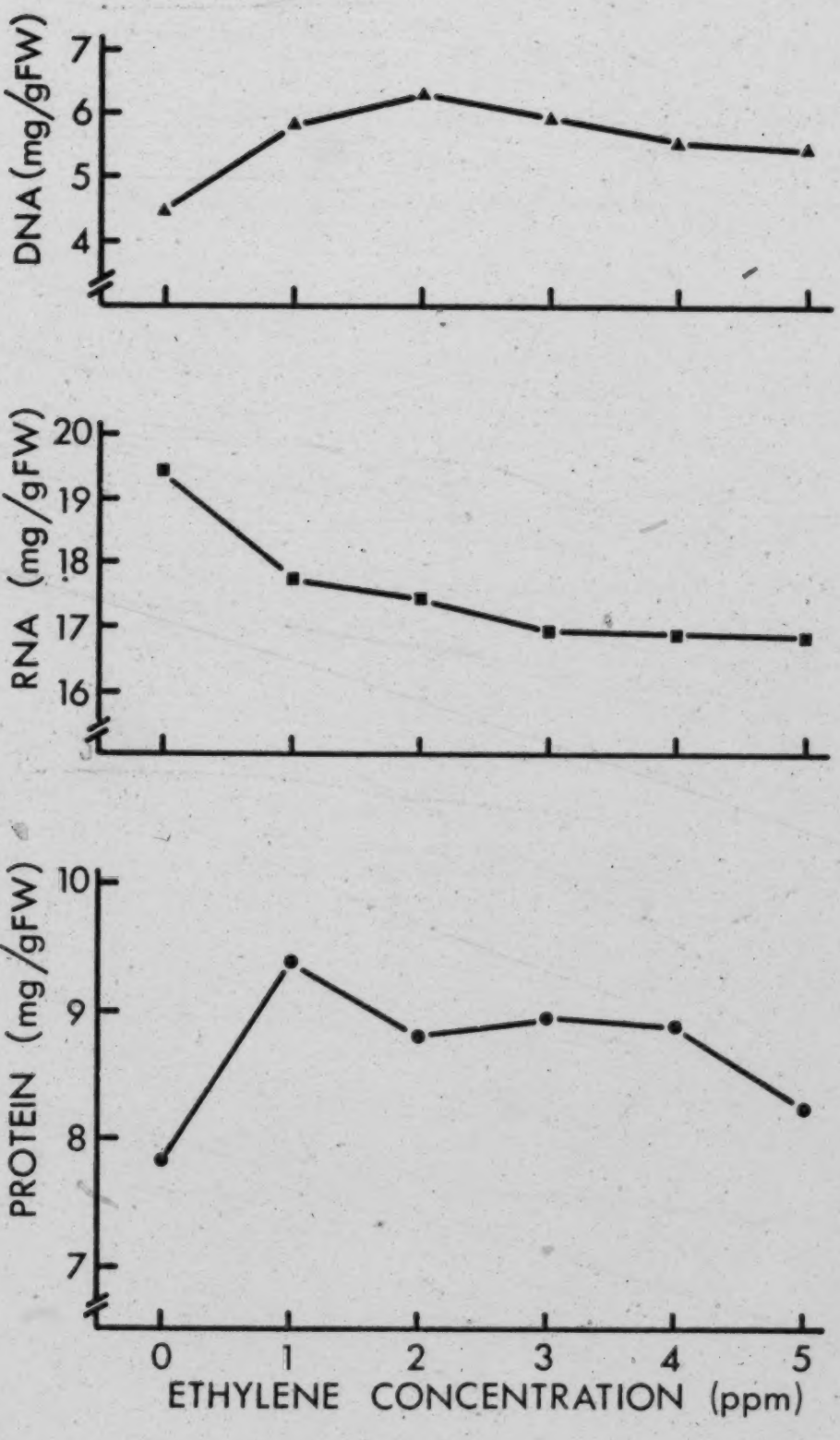


FIGURE 11: The Flow of Information from DNA to Proteins and the Participation of t-RNA in Protein Synthesis.
[Solid arrows represent flow of information, dotted arrows, the involvement of t-RNA and amino acids.]

Although, from the above, ethylene may appear to act at the site of DNA synthesis, the effect of ethylene on RNA should not be overlooked. The results (Figures 8, 9 and 10) showed that ethylene had a pronounced effect on RNA content of the buds. Thus the RNA level of the buds immediately after the ethylene exposure reached a maximum at 2 ppm ethylene concentration, and was maintained at this level even though ethylene concentrations were increased to 5 ppm. This could suggest, that although

FIGURE 10: Effect of Ethylene During the Postharvest Maturation Period

Tulip bulbs (cv. Paul Richter, G-stage) were treated with varying ethylene concentrations for a 4-day period. The figure gives the effect of ethylene concentration on the DNA, RNA and protein levels of the bud two weeks after the ethylene treatment.


DNA (mg/gFW)
7
6
5
4
RNA (mg/gFW)
20
19
18
17
16
PROTEIN (mg/gFW)
10
9
8
7
0
1
2
3
4
5
ETHYLENE CONCENTRATION (ppm)

coding for specific m-RNA had to be achieved prior to increase in the RNA content, once the specific m-RNA was present it was able to provide the necessary function of conveying the message for the required protein. Comparison of the RNA and protein content (Figures 8, 9 and 10) substantiate this view; except for a lag phase, the RNA and protein levels follow similar trends.

The variation in the ratios of the two types of nucleic acids, and nucleic acids and protein caused by ethylene (Tables 3, 4 and 5) substantiates further the hypothesis that ethylene may act at the site of nucleic acid synthesis. The DNA:RNA and DNA:protein ratios of the treated buds showed that these ratios are greater than those in the control. Therefore, relatively higher amounts of DNA were synthesized as compared to RNA and protein in the treated buds.

The RNA:protein ratio of the treated buds, however, showed that these buds contained relatively lower RNA concentrations when compared to protein. This may be explained if it is assumed that in ethylene treated buds there is a requirement for a limited number of specific proteins. For example, in the enhancement of abscission by ethylene, large amounts of cellulase were reported to exist in the abscission zone, and this cellulase contributed significantly to the observed increase in protein content (3, 46). Thus, one or more specific m-RNA could account for the higher protein content.

The percentage difference from control $\left[\frac{\text{treated-control}}{\text{control}} \times 100\right]$ of DNA, RNA and protein varied with the ethylene concentration (Table 6). The concentration of the DNA, immediately after the ethylene exposure, increased with the corresponding increase in ethylene concentrations (Figure 12). This increase was less one week after the exposure and after two weeks the concentration of DNA at the 4 and 5 ppm ethylene concentrations

TABLE 6: Effect of Ethylene During the Postharvest Maturation Period (20 C)

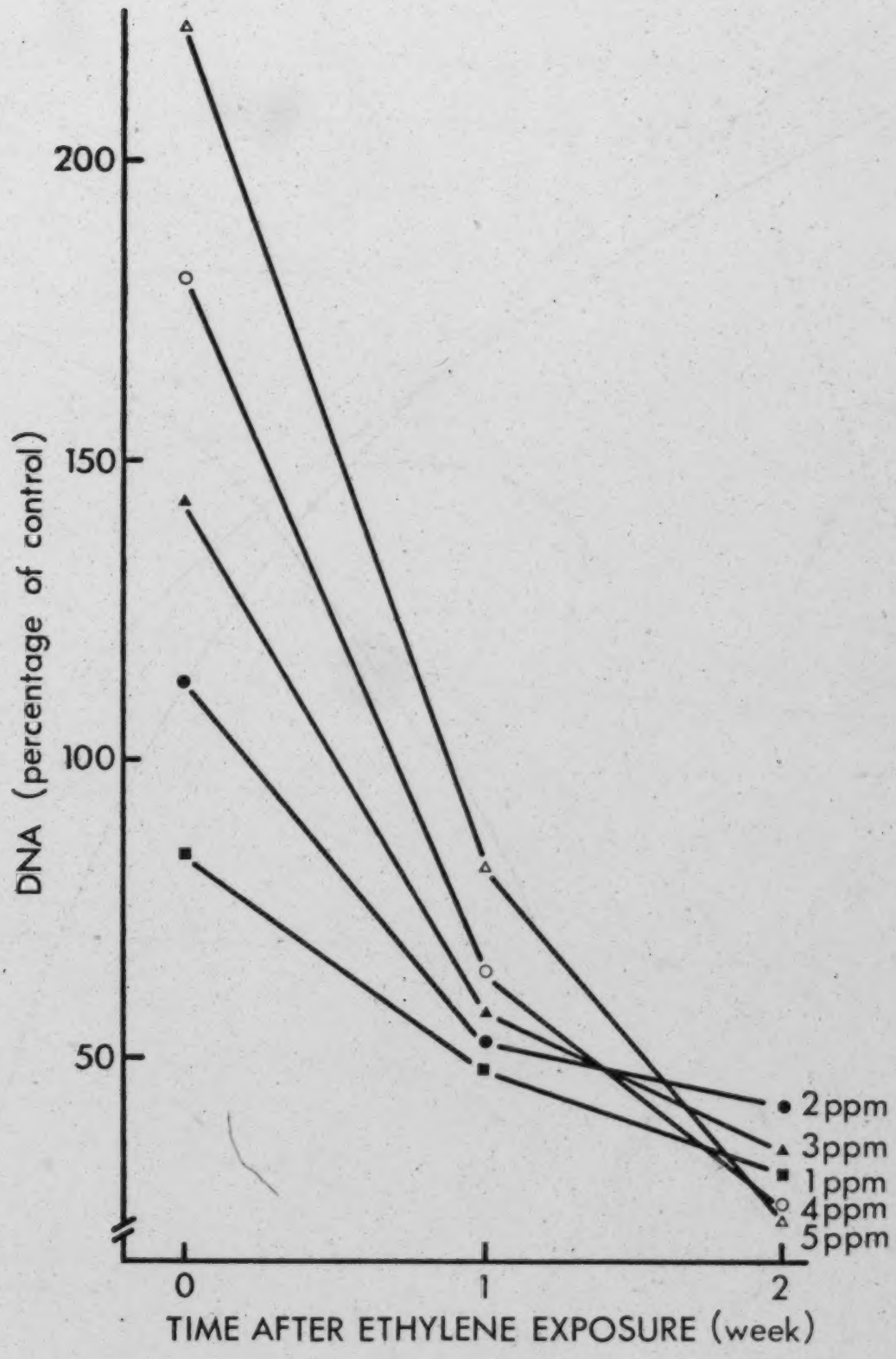
Time After Ethylene Exposure (week)	Ethylene Concentration (ppm) 1	2	3	4	5
DNA (% Control*)					
0	84.0	113.6	143.2	180.0	222.4
1	48.1	53.3	58.5	65.5	82.6
2	31.6	42.7	34.8	25.7	23.7
RNA (% Control*)					
0	34.8	46.0	47.8	48.8	50.5
1	13.4	29.1	20.8	23.8	13.5
2	- 8.3	-10.0	-12.2	-12.4	-12.5
Protein (% Control*)					
0	48.6	69.5	90.7	97.0	121.8
1	49.5	56.4	59.0	50.5	33.9
2	19.7	12.4	14.3	13.8	5.4

Tulip bulbs (cv. Paul Richter, G-stage), in sealed jars and in the dark, were exposed to various concentrations of ethylene for a period of 4 days. The jars were then opened and the buds from these bulbs were analyzed for DNA, RNA and protein immediately, and one and two weeks after the ethylene exposure. The TCA-Acetone method of extraction was employed.

* [(Treated-Control)/Control] x 100

FIGURE 12: Effect of Ethylene During the Postharvest Maturation Period

Tulip bulbs (cv. Paul Richter, G-stage) were treated with varying ethylene concentrations for a 4-day period. The figure gives the effect of removing the ethylene treatment on the percentage difference from control of DNA.


200
150
100
50
DNA (percentage of control)
0
1
2
TIME AFTER ETHYLENE EXPOSURE (week)
2ppm
3ppm
1ppm
4ppm
5ppm

were lower than those at the other concentrations (Figure 12). The concentration of DNA did not show signs of levelling off two weeks after the exposure to ethylene, but, there is the possibility that the DNA content in the treated buds may be lower than that in the control buds after longer periods subsequent to the ethylene exposure. This could explain the delayed effects of ethylene that have been noted (76).

The concentration of RNA, immediately after the ethylene exposure (Figure 13), increased with ethylene concentrations. This increase, however, was not proportional to the ethylene concentration; the concentrations of RNA in the buds receiving the 2, 3, 4 and 5 ppm ethylene concentrations were almost similar, while that of the 1 ppm was much lower. The content of RNA in treated buds decreased with time after the ethylene treatment; the decrease was greatest with the highest ethylene concentration (5 ppm) over the first week.

As with DNA and RNA, the protein content of the treated buds increased with increasing ethylene concentrations (Figure 14). The concentration of protein decreased with time after the ethylene treatment, however, unlike either DNA or RNA, the decrease was greatest between the second and third week after the exposure for all concentrations of ethylene employed with the exception of the 4 and 5 ppm concentrations. The decrease in these two instances was greatest in the first week after the exposure. Two weeks after the exposure to ethylene the concentration of protein in buds which received the higher concentrations gave lower values. This could explain the delayed effects of ethylene (76).

No 'open buds' (Figure 2) were observed immediately after the 4-day ethylene exposure. Nevertheless, signs of open buds were observed one week after the treatment. The bulbs which received the 4 and 5 ppm ethylene concentrations during the 4-day exposure period, had stamens which

FIGURE 13: Effect of Ethylene During the Postharvest Maturation Period

Tulip bulbs (cv. Paul Richter, G-stage) were treated with varying ethylene concentrations for a 4-day period. The figure gives the effect of removing the ethylene treatment on the percentage difference from control of RNA.

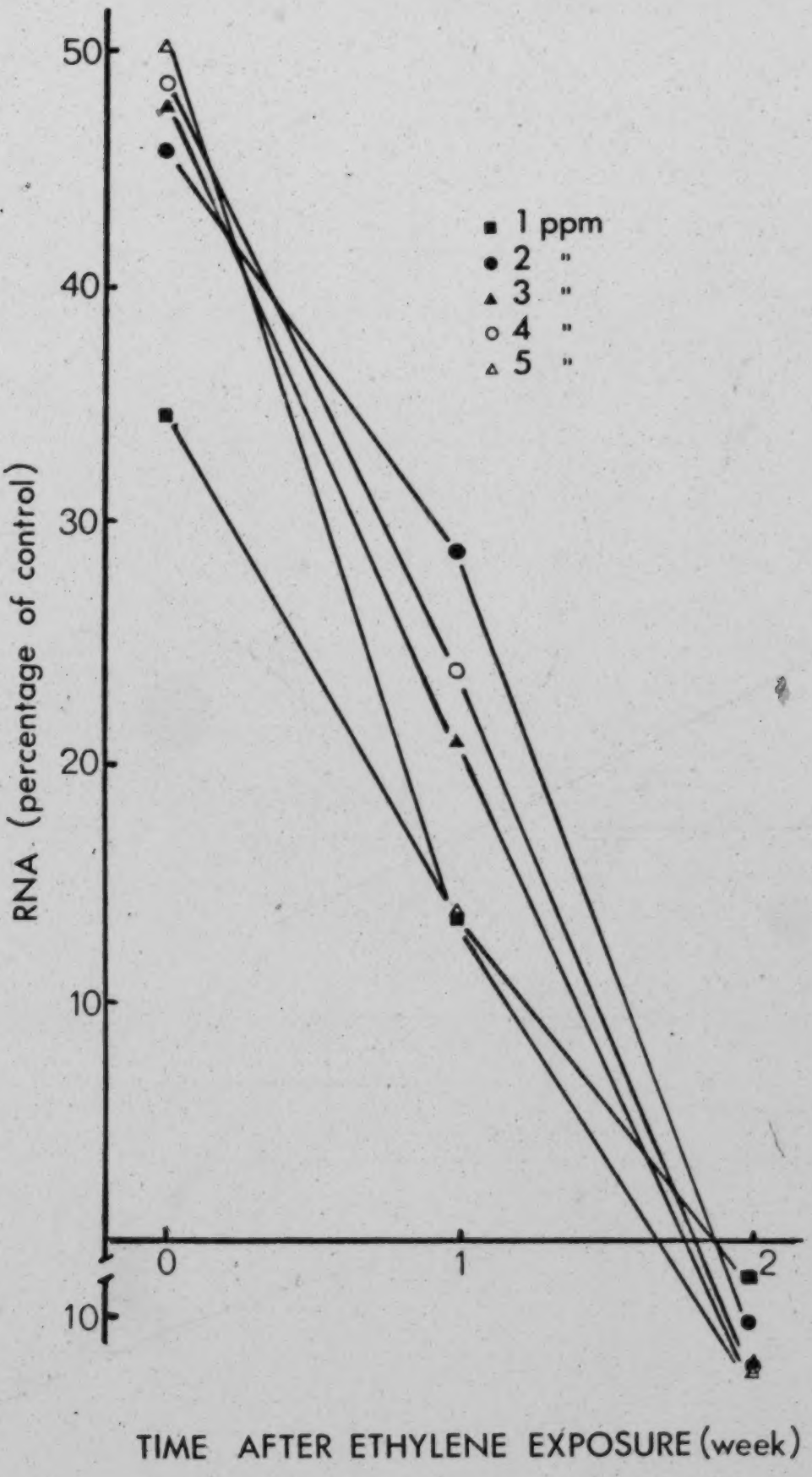
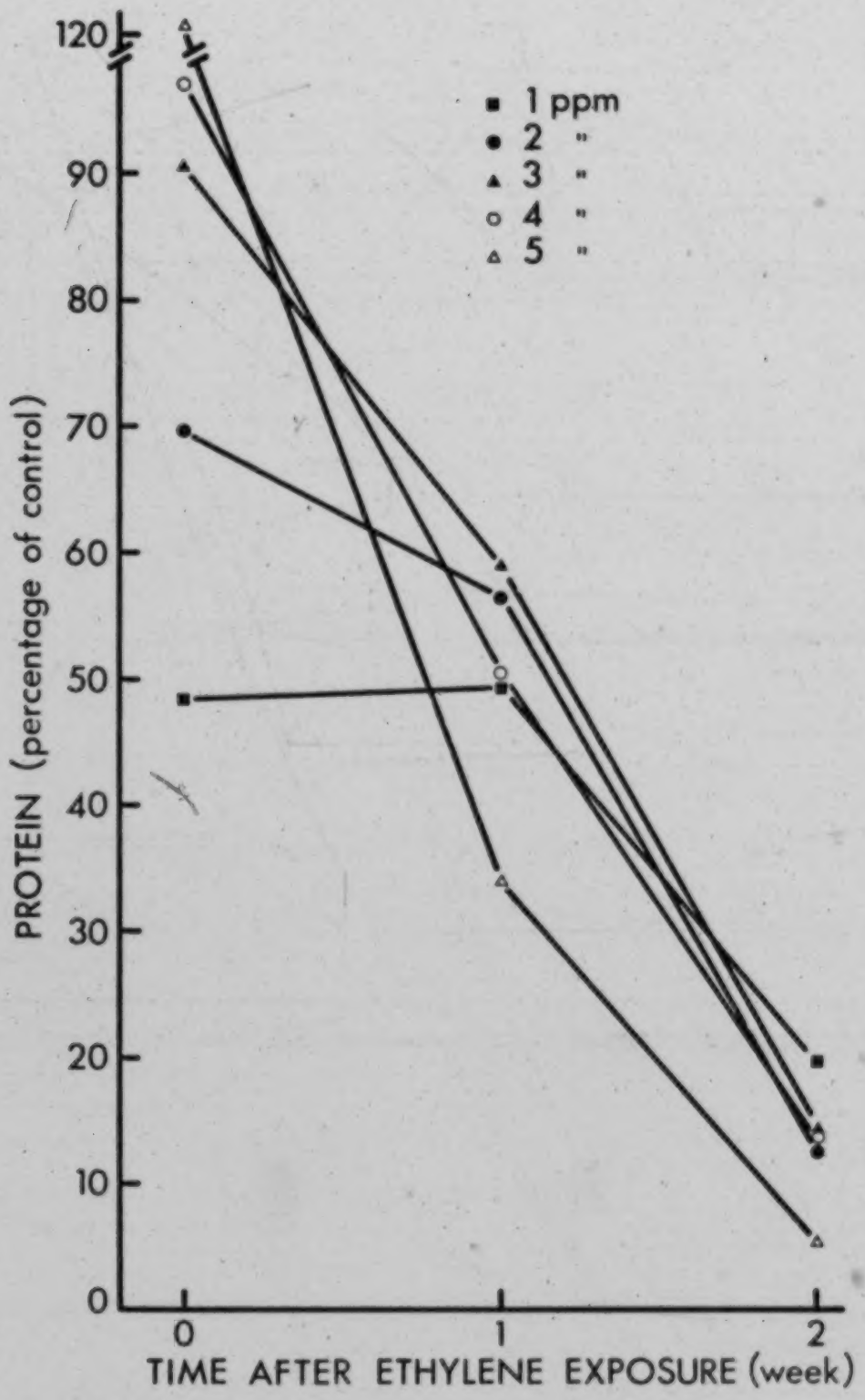

50
40
30
20
10
10
0
1
2
RNA (percentage of control)
1 ppm
2 "
3 "
4 "
5 "
TIME AFTER ETHYLENE EXPOSURE (week)

FIGURE 14: Effect of Ethylene During the Postharvest Maturation Period

Tulip bulbs(cv. Paul Richter, G-stage) were treated with varying ethylene concentrations for a 4-day period. The figure gives the effect of removing the ethylene treatment on the percentage difference from control of protein.


120
90
80
70
60
50
40
30
20
10
0
PROTEIN (percentage of control)
1 ppm
2 "
3 "
4 "
5 "
0
1
2
TIME AFTER ETHYLENE EXPOSURE (week)

were slightly longer than the tepals. Two weeks after the exposure buds from the 2 and 3 ppm concentrations were in a similar condition. The apparent lag phase experienced prior to the formation of the open buds is another of the delayed effect of ethylene. There is, however. the possibility that open buds may be observed immediately after the ethylene treatment. Exposures of bulbs to higher ethylene levels than those used here or for longer periods may give buds that are open immediately after the treatment is removed. Although the buds were opened, bud necrosis did not result; this may have been due to the absence of mites (Figure 15).

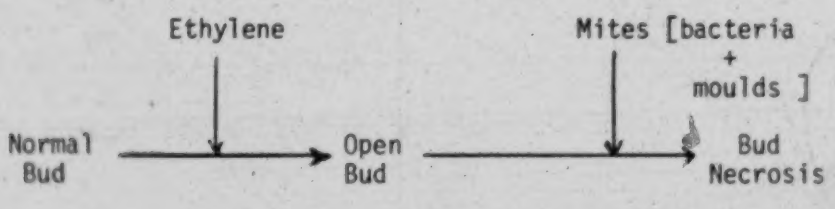


FIGURE 15: Schematic Representation of the Hypothesis Concerning the Ontogency of Bud Necrosis (73).

When the flowers came into bloom, blasting of the flower-buds were noted. The degree of blasting was slight; the stamens only were blasted (Figure 16.1), no severe blasting occurred (Figure 16.2). A prolonged exposure to these ethylene concentrations could probably give higher degrees of flower-bud blasting. With the exception of flower-bud blasting and general reduction of flower height and size, no other abnormalities were apparent in the treated plants.

2. Effect of Ethylene During the Cooling Period (10 C)

Two sets of experiments were carried out during this period:-

a. the effect of ethylene following exposure ('Recovery Studies'),

FIGURE 16.1: Effect of Ethylene During the Postharvest Maturation Period

Tulip bulbs (cv. Paul Richter, G-stage) were treated with varying ethylene concentrations for a 4-day period. The figure shows a mild form of blasting obtained with ethylene treatment during this period. Only the stamens of the flowers were blasted, when the treated buds came into bloom.

FIGURE 16.2: Effect of Ethylene During the Forcing Period

Tulip plants (cv. Paul Richter) were treated with ethylene for 2, 7 and 10 days. Severe flower-bud blasting was observed in the treatment of the 0.3 and 0.5 ppm ethylene concentrations, when the exposure periods were 7 days or longer. In this form of blasting the flower stalk does not elongate and the bud remains between the folds of the leaves, the bud appears white and papery.

1.
Stamen Blasting

2.
Severe Flower-Bud Blasting

b. the effect of different lengths of ethylene exposures.

a. The Effect of Ethylene Following Exposure

This study was conducted using the bulbs (cv. Paul Richter) received from the commercial supplier. These bulbs when received, had reached the G-stage (Figure 1). A portion of the bulbs (Group A; Materials and Methods) were placed in cold storage (10 C) and treated with ethylene.

Bulbs, exposed for 2 weeks to 0, 5 and 10 ppm ethylene concentrations, were analyzed immediately after the ethylene treatment and then 2 and 3 weeks after the exposure. The results are given in Table 7.

Bulbs, exposed for 4 weeks to 0, 5 and 10 ppm ethylene concentrations, were analyzed immediately after the exposure and subsequent analyses were carried out 1, 2 and 3 weeks after the removal of the treatment. These results are presented in Table 8.

The results indicate that the RNA content of the buds fell with increasing ethylene concentrations immediately after the ethylene treatment. Nevertheless, the buds 'recovered' and after 2 weeks following the treatment the RNA content of the treated buds were higher than those of the controls. This is illustrated for the 4-week exposure in Figure 17. From this, it is seen that after a lag phase the RNA content of treated buds rose. This lag phase was more pronounced at the higher ethylene treatment (10 ppm).

Protein content in treated buds immediately after the ethylene exposure was higher in the treated buds, the protein content of the bud which received the higher ethylene concentration giving the higher value (Tables 7 and 8). The protein levels one week after the ethylene treatment were higher in the control buds, the higher ethylene treatment (10 ppm) giving the lowest value (Table 8). This decline in the protein content corresponded to the decline in the RNA content (Figure 17). This finding

TABLE 7: Effect of Ethylene During the Cooling Period (10 C)

	Time After Ethylene Exposure (week)	Ethylene Concentration (ppm) 0	5	10
RNA (mg/g F.W.)				
	0	14.48	14.28	14.14
	2	17.75	23.29	20.91
	3	10.90	15.21	11.59
Protein (mg/g F.W.)				
	0	22.77	23.79	25.43
	2	23.53	27.58	24.75
	3	22.95	28.28	25.20

Tulip bulbs (cv. Paul Richter, G-stage), in sealed jars and in the dark, were exposed to 0, 5 and 10 ppm ethylene for two weeks. The jars were then opened and the floral buds analyzed for RNA and protein immediately, and two and three weeks after the ethylene exposure. The TCA-Acetone method of extraction was employed.

TABLE 8: Effect of Ethylene During the Cooling Period (10 C)

	Time After Ethylene Exposure (week)	Ethylene Concentration (ppm) 0	5	10
RNA (mg/g F.W.)				
	0	28.18	19.19	18.06
	1	17.90	13.06	12.47
	2	19.44	22.86	18.78
	3	17.92	21.48	19.74
RNA (% Control*)				
	0		-17.21	-22.09
	1		-27.04	-30.34
	2		+17.59	- 3.40
	3		+19.87	+10.16
Protein (mg/g F.W.)				
	0	27.41	29.76	31.12
	1	38.12	36.29	35.14
	2	35.68	50.88	39.70
	3	31.00	45.09	37.55
Protein (% Control*)				
	0		+ 8.57	+ 3.54
	1		- 4.80	- 7.82
	2		+42.60	+11.27
	3		+45.45	+21.13
RNA:Protein				
	0	0.85	0.64	0.58
	1	0.47	0.36	0.35
	2	0.54	0.45	0.47
	3	0.58	0.48	0.53

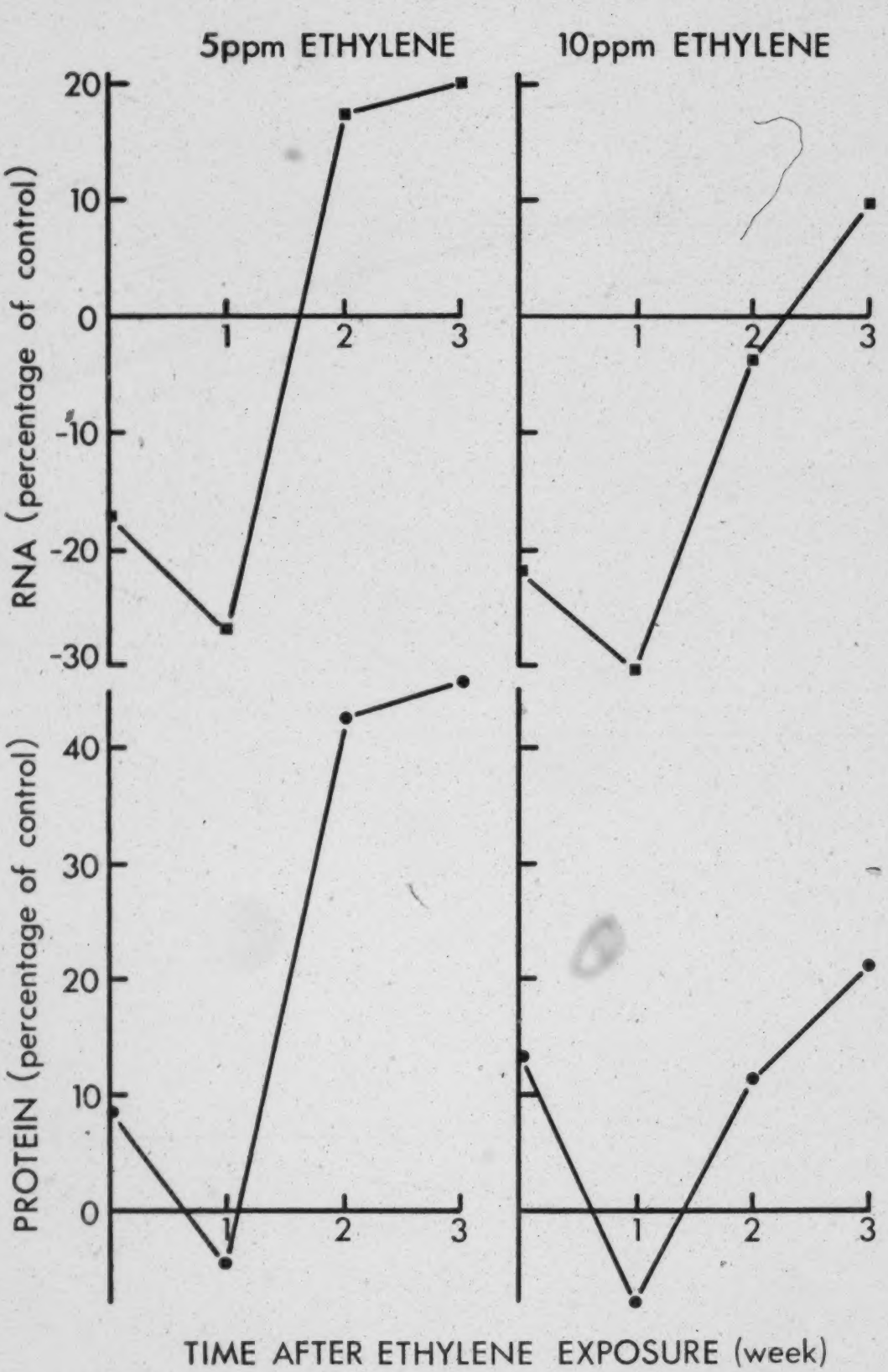
Tulip bulbs (cv. Paul Richter, G-stage), in sealed jars and in the dark, were exposed to 0, 5 and 10 ppm ethylene for four weeks. The jars were then opened and the floral buds analyzed for RNA and protein immediately, and one, two and three weeks after the ethylene exposure. The TCA-Acetone method of extraction was employed.

* [(Treated-Control)/Control] x 100

FIGURE 17: Effect of Ethylene During the

Cooling Period

Tulip bulbs (cv. Paul Richter, G-stage) were treated with varying ethylene concentrations for 4 weeks. The figure gives the effect of the removal of the ethylene treatment on the levels of RNA and protein at the 5 and 10 ppm ethylene concentrations.


5ppm ETHYLENE
10ppm ETHYLENE
RNA (percentage of control)
PROTEIN (percentage of control)
20
10
0
-10
-20
-30
40
30
20
10
0
1
2
3
TIME AFTER ETHYLENE EXPOSURE (week)

was in agreement with the schematic representation of protein synthesis (Figure 11). Since the action of ethylene was apparent on both RNA and protein content, the results indicate that ethylene may act at some point before or at the site of RNA synthesis.

No floral abnormalities were caused by the treatment. When the flowers came into bloom no abnormalities, other than a slight reduction in flower size and height were noted. This demonstrates that the delayed effects of ethylene is non-functional during the cooling period. The lack of abnormalities is an indication that bulbs during this period are either insensitive to ethylene or that they can 'recover' from the ethylene treatment. The difference in the RNA and protein content of treated and control buds sopports the latter view. An examination of the RNA:protein ratios (Figure 18) showed that although the ratios were very different immediately after the ethylene exposure (the higher concentration (10 ppm) giving a lower ratio), the ratios eventually approached a common value some time after the ethylene treatment. This substantiates the hypothesis that the buds were able to 'recover' from ethylene treatment during this period; and could explain the absence of floral abnormalities when bulbs are subjected to ethylene concentrations (even up to 100 ppm) during the cooling period (76).

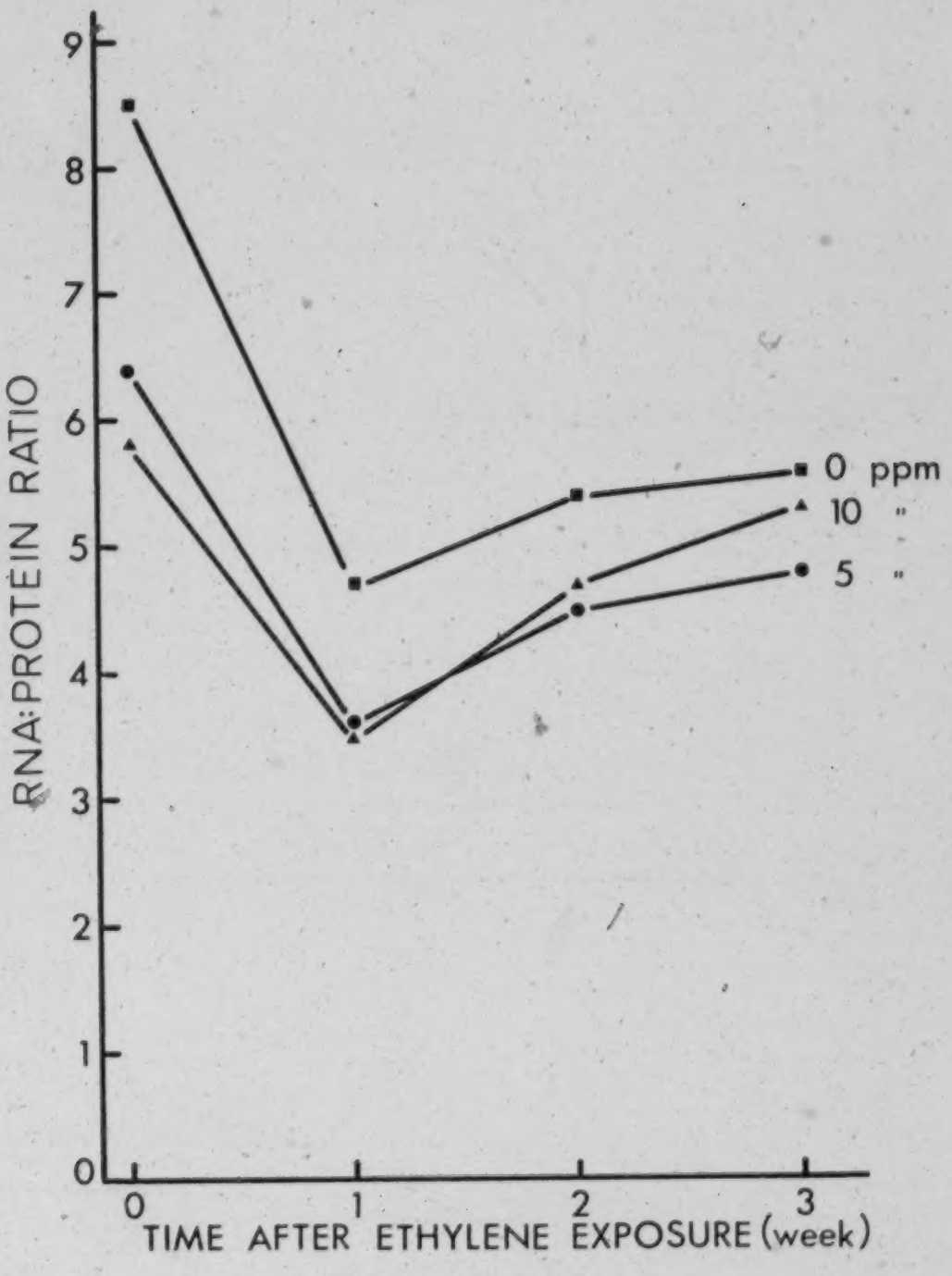
b. The Effect of Different Lengths of Ethylene Exposures

The first generation bulbs (cv. Paul Richter) obtained from the commercially supplied bulbs grown in the cold frame (Group B; Materials and Methods) were employed in this study. The bulbs were graded prior to use, only bulbs (11-12 cm) free of blemishes and from plants with normal flowers were selected.

When flower formation of these bulbs was completed (G-stage, Figure 1), the bulbs were placed in cold (10 C) storage and treated with

FIGURE 18: Effect of Ethylene During the Cooling Period

Tulip bulbs (cv. Paul Richter, G-stage) were treated with varying concentrations of ethylene for 4 weeks.
The figure gives the effect of the removal of the ethylene treatment on the RNA:protein ratio, for the 0, 5 and 10 ppm ethylene concentrations.


RNA:PROTEIN RATIO
9
8
7
6
5
4
3
2
1
0
0
1
2
3
TIME AFTER ETHYLENE EXPOSURE (week)
0 ppm
10 "
5 "

0.5 ppm ethylene for 1, 2, 3, 4 and 5 weeks. The buds were analyzed immediately after the treatment and again one week later. The results are given in Tables 9 and 10 respectively. As growth was proceeding during the treatment period, the levels of the nucleic acids and protein, fluctuated with the stage of growth. Thus for a more valid comparison the results were expressed as 'percentage difference from control' which is given by:-

$$\frac{\text{treated - control}}{\text{control}} \times 100.$$

Table 9 and Figure 19 give the results for DNA, RNA and protein expressed as percentage difference from control subsequent to the ethylene exposures. One week after the ethylene treatment at 0.5 ppm, the DNA content in treated buds was significantly higher than that in the control. The difference in the DNA content, nevertheless, decreased markedly with the longer ethylene exposures. This may have been due to the following factors:-

a. the autocatalytic action of ethylene,

   [At low concentrations, ethylene behaves as a growth hormone and stimulates the synthesis of DNA (53). Longer exposures at these concentrations, however, causes the production of endogenous ethylene (2, 10, 16). Thus the longer ethylene exposures give similar effects as the higher concentrations - where the action is no longer stimulatory, but, inhibitory; thus resulting in lower DNA synthesis.]

b. the building up of resistence, by the plant, to ethylene, thus resulting in a less pronounced difference between the DNA levels in treated and control buds.

Either one or perhaps both of these factors may have contributed to the lower difference between the DNA content of the treated and control buds.

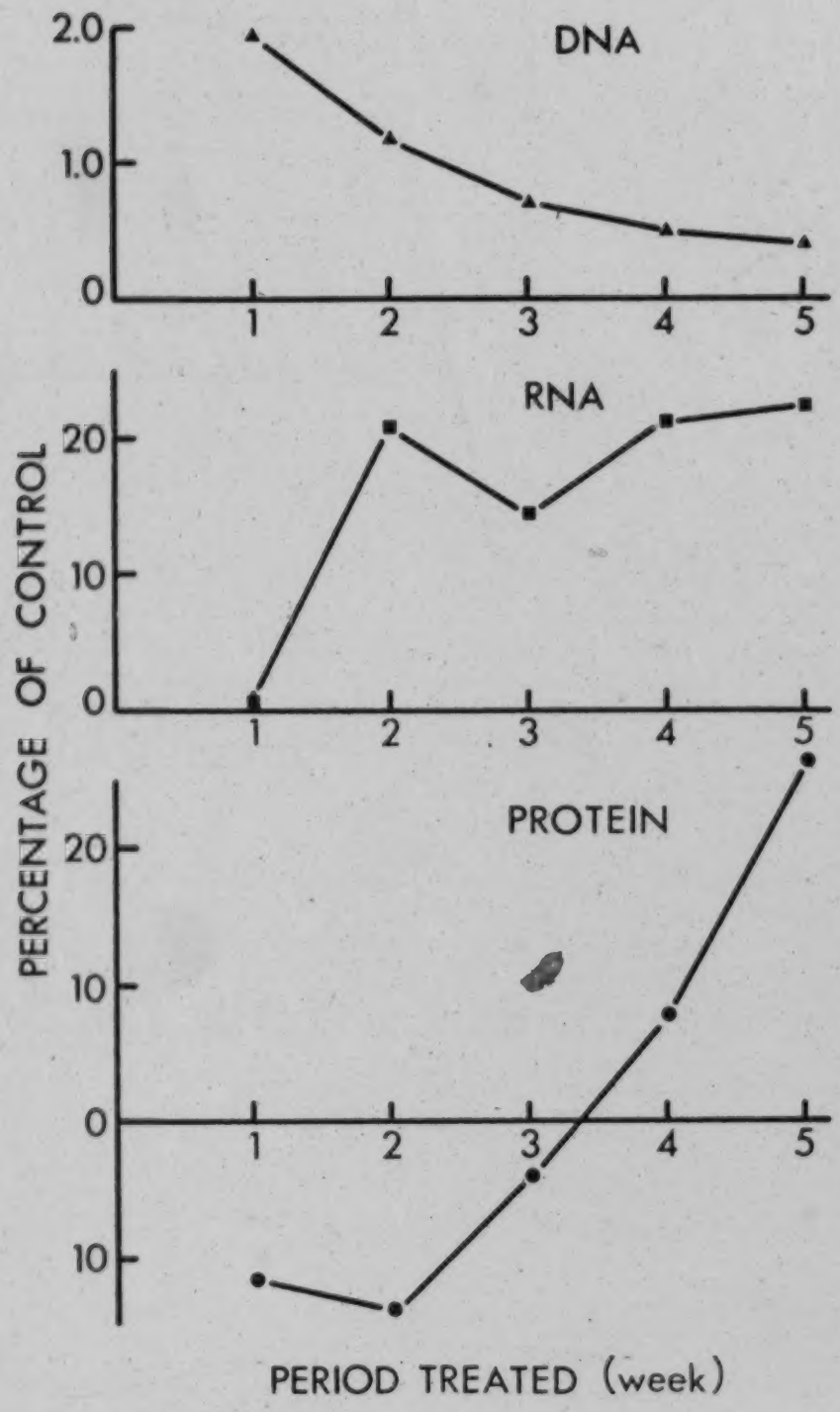
TABLE 9: Effect of Ethylene During the Cooling Period (10 C)

	Length of Treatment (week)	DNA (mg/g F.W.)	RNA (mg/g F.W.)	Protein (mg/g F.W.)
Control (0 ppm Ethylene)				
	0	5.71	22.92	12.43
	1	5.19	14.79	12.48
	2	5.17	14.38	14.16
	3	4.28	15.96	13.27
	4	4.21	14.83	11.48
	5	3.95	17.40	14.75
0.5 ppm Ethylene				
	1	5.29	14.92	11.04
	2	5.23	17.37	11.22
	3	4.31	18.25	12.76
	4	4.23	17.98	12.40
	5	3.96(5)	21.31	18.69
Percentage Difference from Control [(Treated-Control)/Control] x 100				
	1	1.93	0.88	-11.54
	2	1.16	20.79	-13.70
	3	0.70	14.35	- 3.84
	4	0.48	21.24	+ 8.01
	5	0.38	22.47	+26.71

Tulip bulbs (cv. Paul Richter, G-stage), in sealed jars and in the dark were exposed to 0.5 ppm ethylene for various periods. The floral buds from these bulbs were analyzed for DNA, RNA and protein immediately after the ethylene exposure. The TCA-Acetone method of extraction was employed.

FIGURE 19: Effect of Ethylene During the Cooling Period

Bulbs (cv. Paul Richter, G-stage) were treated with 0.5 ppm ethylene during the cooling period for varying lengths of time. The figure gives the effect of length of exposure period on the levels of DNA, RNA and protein, immediately after the removal of the ethylene.


DNA
2.0
1.0
0
1
2
3
4
5
RNA
20
10
0
PROTEIN
20
10
0
10
PERCENTAGE OF CONTROL
PERIOD TREATED (week)

The RNA levels of treated buds increased rapidily with the length of the ethylene exposure. This value, however, levelled off after the second week of treatment. (Figure 19). A similar trend was noted with bulbs treated during the postharvest maturation period with different concentrations of ethylene (0, 1, 2, 3, 4 and 5 ppm ethylene for a 4-day period) - the RNA content increased before levelling off at the higher ethylene concentrations (Figure 14). This further serves to substantiate the hypothesis that the longer ethylene treatments, at low concentrations, could give similar results as high concentrations of ethylene extending over shorter periods.

After an initial lag phase, the protein levels of treated buds increased with the length of ethylene treatment. During the lag phase the protein content in the treated buds was lower than that found in the control (Figure 19). This decline in protein levels prior to the increase could explain some of the conflicting results that have appeared in the literature concerning the action of ethylene on protein synthesis in plants or plant parts (2).

The combination of the results illustrated in Figure 19 substantiates the idea that the action of ethylene may be at the site of DNA synthesis. Ethylene stimulated DNA synthesis prior to the synthesis of RNA and protein. The increase in the content of the latter two components would follow once DNA synthesis was stimulated (Figure 11). This indicated that the coding for the ultimate effects of ethylene had to be satisfied before the other changes were apparent.

The results obtained in this experiment dealing with RNA and protein content is in agreement with the results obtained in an earlier experiment on the effect of ethylene during the postharvest maturation period. The RNA content, in both these instances, did not increase with

the corresponding increases in the protein content. As in the earlier experiment, once the specific m-RNAs were present they could account for the increases in the protein content (Figures 14 and 19).

Table 10 and Figure 20 give the results for the percentage difference from control of DNA, RNA and protein one week after the removal of the ethylene treatments. The greatest difference in the DNA levels was noted in buds which received the longest ethylene exposure (5 weeks). This could mean that ethylene, functioning as a stressing agent (inhibiting the natural metabolic processes of the plant), exerted the greatest stress with the longer exposures. Once the stress(ethylene) was removed (opening of the jars) the greatest relief was experienced after the longest treatment. This relief was then expressed by the sudden and rapid upsurge in DNA synthesis. Hence the DNA levels of the treated buds showed a marked increase with the increase in the length of exposure. If the increase in the concentration of DNA in the treated buds were due to the relief in the stress caused by ethylene, then this value should decrease eventually after the ethylene treatment is removed. There is every possibility that this actually occurred, because no adverse effects of the ethylene treatment were apparent in the flowers of the treated bulbs when they came into bloom.

The RNA levels in treated buds, one week after the removal of the treatment, showed an initial high level before declining to a lower constant value in the buds which had received the 3-week exposure (Figure 20). This may be explained on the grounds of the delay between the increase in the DNA and RNA levels. As seen from Figure 19, the RNA concentration was lower when the concentration of DNA was high. This same relationship still persisted one week after the removal of the ethylene treatment (Figure 20).

One week after the removal of the ethylene treatment the

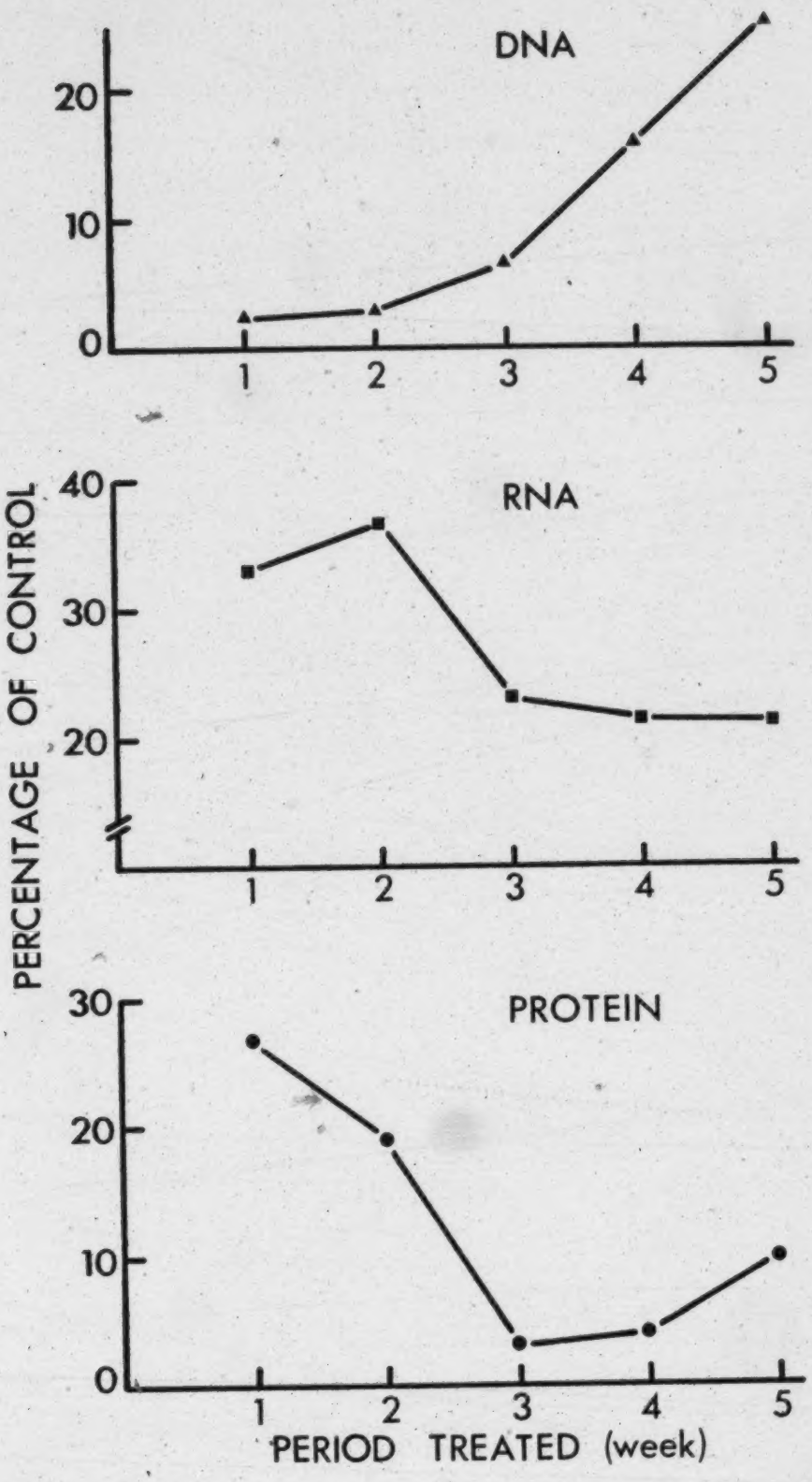
TABLE 10:- Effect of Ethylene During the Cooling Period (10 C)

	Length of Treatment (week)	DNA (mg/g F.W.)	RNA (mg/g F.W.)	Protein (mg/g F.W.)
Control (0 ppm Ethylene)				
	0	5.71	22.92	12.43
	1	5.17	17.37	11.22
	2	4.28	15.96	13.27
	3	4.21	14.83	11.48
	4	3.95	17.40	11.45
	5	3.42	15.87	10.03
0.5 ppm Ethylene				
	1	5.30	23.12	14.19
	2	4.40	21.78	15.79
	3	4.49	18.25	11.82
	4	4.58	12.12	15.32
	5	4.28	19.26	11.03
Percentage Difference from Control [(Treated-Control)/Control] x 100				
	1	2.51	33.10	26.47
	2	2.80	36.47	18.99
	3	6.65	23.06	2.96
	4	15.95	21.38	3.86
	5	25.15	21.36	9.97

Tulip bulbs (cv. Paul Richter, G-stage), in sealed jars and in the dark were exposed to 0.5 ppm ethylene for various periods. The floral buds from these bulbs were analyzed for DNA, Rna and protein one week after the ethylene exposure. The TCA-Acetone method of extraction was employed.

FIGURE 20: Effects of ethylene During the Cooling Period

Bulbs (cv. Paul Richter, G-stage) were treated with 0.5 ppm ethylene during the cooling period for varying lengths of time. The figure gives the effect of length of exposure period on the levels of DNA, RNA and protein, one week after the removal of the ethylene.


DNA
20
10
0
1
2
3
4
5
RNA
40
30
20
PERCENTAGE OF CONTROL
PROTEIN
30
20
10
0
PERIOD TREATED (week)

same relationship existed between the protein levels in treated buds and the DNA levels of these buds; high levels of DNA corresponded to low levels of protein (Figures 19 and 20). This apparent contridiction may be explained on the basis of the time difference between DNA synthesis and protein synthesis (Figure 11). Thus after an increase in the DNA content, there is a lag phase before the increase in protein content is apparent.

As in the case of the former treatment (Effect of Ethylene During the Cooling Period - bulbs treated for 2 and 4 weeks to different ethylene concentrations), There were no abnormalities in the floral buds after the treatment. In addition no delayed effects of the ethylene treatment were apparent when the flowers of these bulbs came into bloom. This illustrates that bulbs during the cooling period are either insensitive to ethylene or are able to 'recover' from ethylene treatments.

3. Effect of Ethylene During the Forcing Period (Day Temperature 18 C, Night Temperature 16 C)

The first generation bulbs (cv. Paul Richter) obtained from the commercially supplied bulbs grown in the cold frame were employed in this study (Group B; Materials and Methods). The bulbs were graded after the harvest, only bulbs (11-12 cm) free of blemishes and from plants which had normal flowers were selected. These bulbs were maintained at 20 C until the G-stage was reached, then they were potted and placed in cold (10 C) storage for a 12-week period (Cooling period, refer Materials and Methods). When these plants were ready for forcing they had reached an average height of 20 cm; the buds were, however, still enclosed within the last foliage leaf which had not yet unfolded. The plants were treated during this period to relatively low concentrations of ethylene because the flowers are apparently very susceptible to the gas during this period.

The result of exposing the plants to 0, 0.1, 0.3 and 0.5 ppm ethylene for periods of 2, 7 and 10 days is given in Table 11 and illustrated in Figures 21, 22, 23 and 24. Figures 21 and 22 give the results expressed as mg (nucleic acid or protein)/g F.W., while Figures 23 and 24 give the results expressed on the basis of percentage difference from control of the respective components.

a. Effect on DNA

The results of this experiment showed that the effect of ethylene on the DNA levels of the buds varied with ethylene concentration and length of ethylene treatment.

DNA levels of the buds increased with increasing ethylene concentration (up to 0.5 ppm) after a 2-day exposure period. This relationship, however, was lost with the longer exposures - 7 and 10 days. In the 7-day and 10-day ethylene treatments the DNA levels in buds declined at the 0.3 ppm ethylene concentration. This decrease, in DNA, was greatest in the 10-day exposure (Figures 21 and 23).

The effect of length of ethylene exposure on DNA levels of the buds is shown in Figures 22 and 24. At a concentration of 0.1 ppm ethylene, DNA content increased with increasing length of exposure. At the ethylene concentrations of 0.3 and 0.5 ppm, the DNA concentration in treated buds started to decline after the 7-day exposure. The decline was gradual at the 0.3 ppm ethylene, but sharp at the higher concentration (0.5 ppm). The DNA levels of all the treated buds were higher than those of the control. Nevertheless, there is a likelihood that the DNA levels of the buds, treated with either higher ethylene concentrations or for longer periods, could be less than that of the control.

In addition, these results substantiates the hypothesis that ethylene at low concentrations, over an extended period, may give effects

TABLE 11: Effect of Ethylene During the Forcing Period (Day Temp. 18 C, Night Temp. 16 C)

Ethylene Concentration (ppm)	DNA		RNA		Protein	
	mg/g F.W.	%Control	mg/g F.W.	%Control	mg/g F.W.	%Control
Day-2						
0	1.40		8.18		13.32	
0.1	1.82	30.00	15.18	85.57	14.41	8.18
0.3	2.03	45.00	15.67	91.56	16.89	26.80
0.5	2.34	67.14	16.13	97.19	17.78	33.48
Day-7						
0	4.47		12.11		12.89	
0.1	6.36	42.28	14.24	17.59	13.42	4.11
0.3	7.99	78.74	15.50	27.99	15.59	20.95
0.5	7.67	71.59	14.77	21.97	15.73	22.03
Day-10						
0	4.09		11.48		12.71	
0.1	6.88	68.22	13.17	14.72	13.19	3.78
0.3	7.26	77.51	14.67	27.79	14.97	17.78
0.5	5.98	46.21	11.89	3.57	14.60	14.87

Tulip plants (cv. Paul Richter) after the cooling period (12 weeks at 10 C) were treated, in sealed plastic cabinets, during forcing with 0, 0.1, 0.3 and 0.5 ppm ethylene for 2, 7 and 10 days. The floral buds were analyzed immediately after the ethylene exposure for DNA, RNA and protein. The TCA-Acetone method of extraction was employed.

FIGURE 21: Effect of Ethylene During the Forcing Period

Tulip plants (cv. Paul Richter) were exposed to varying ethylene concentrations and for different lengths of time.
The figure gives the effect of ethylene concentration on the DNA, RNA and protein levels (mg/g F.W.).

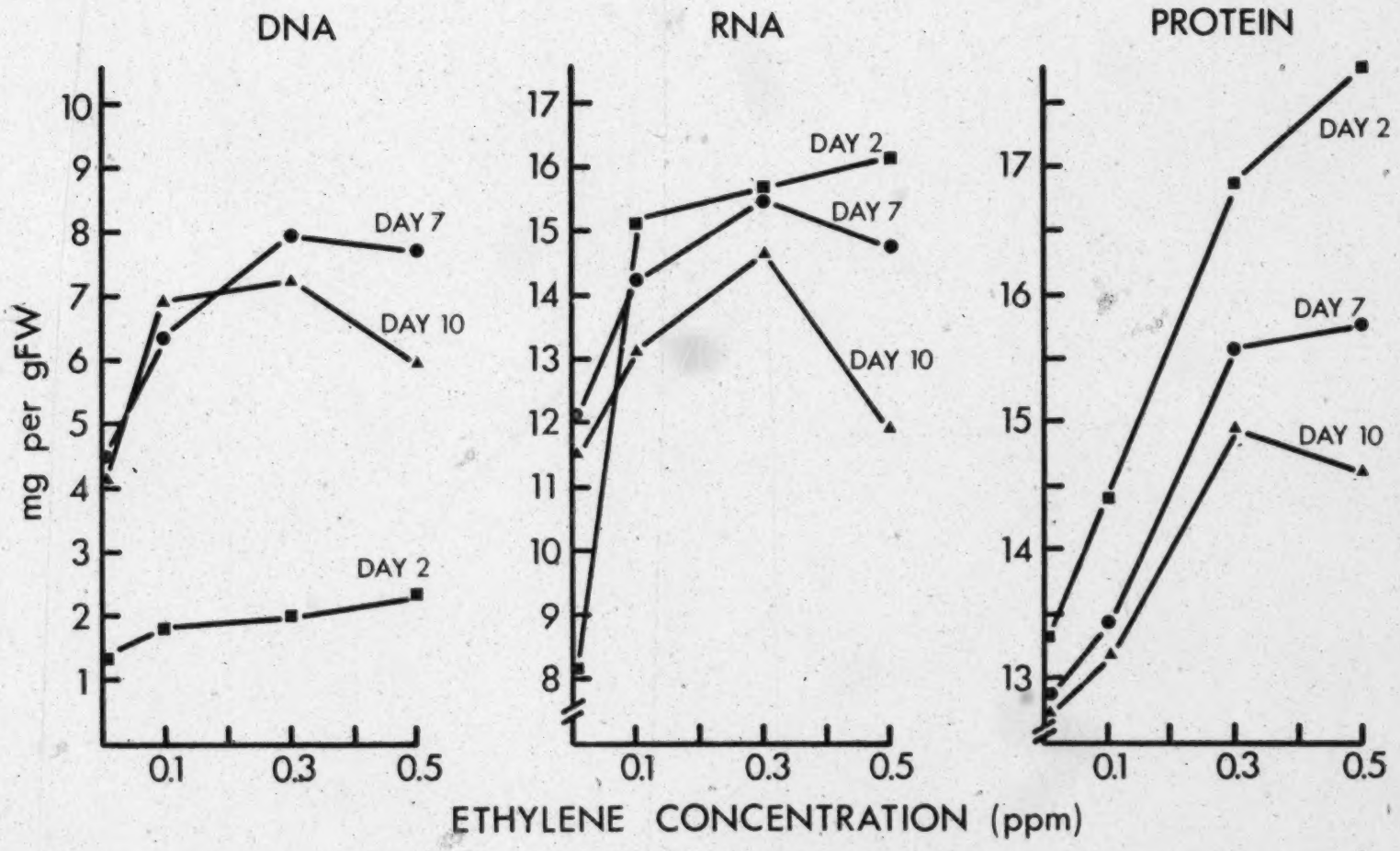

DNA
RNA
PROTEIN
mg per gFW
DAY 2
DAY 7
DAY 10
ETHYLENE CONCENTRATION (ppm)
0.1
0.3
0.5

FIGURE 22: Effect of Ethylene During the

Forcing Period

Tulip plants (cv. Paul Richter) were exposed to varying ethylene concentrations and for different lengths of time.
The figure gives the effect of the length of exposure on the DNA, RNA and protein levels (mg/g F.W.).

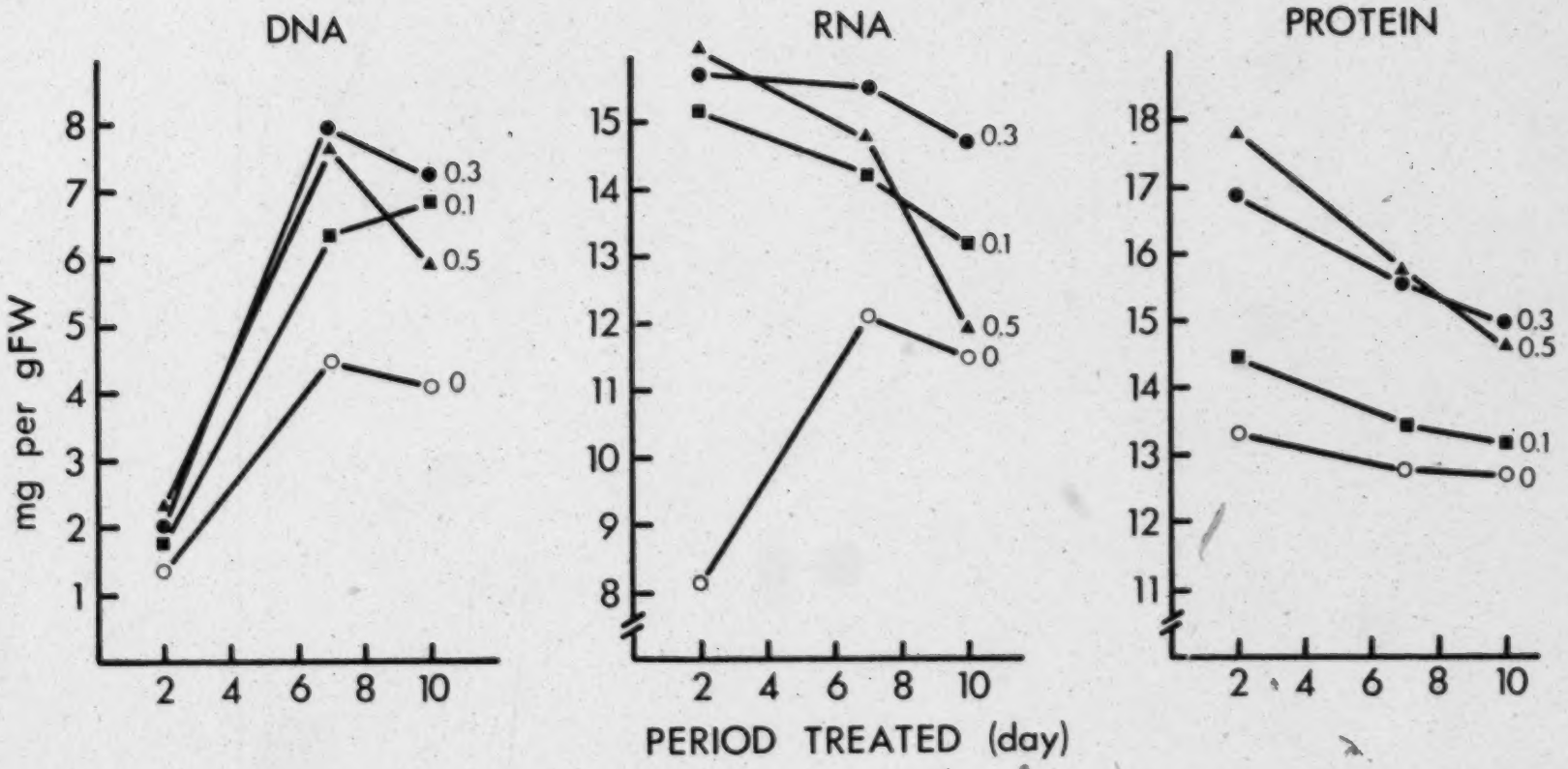

DNA
RNA
PROTEIN
mg per gFW
PERIOD TREATED (day)
0.3
0.1
0.5
0

FIGURE 23: Effect of Ethylene During the

Forcing Period

Tulip plants (cv. Paul Richter) were exposed to varying ethylene concentrations and for different lengths of time.
The figure gives the effect of ethylene concentration of the DNA, RNA and protein levels (percentage difference from control).

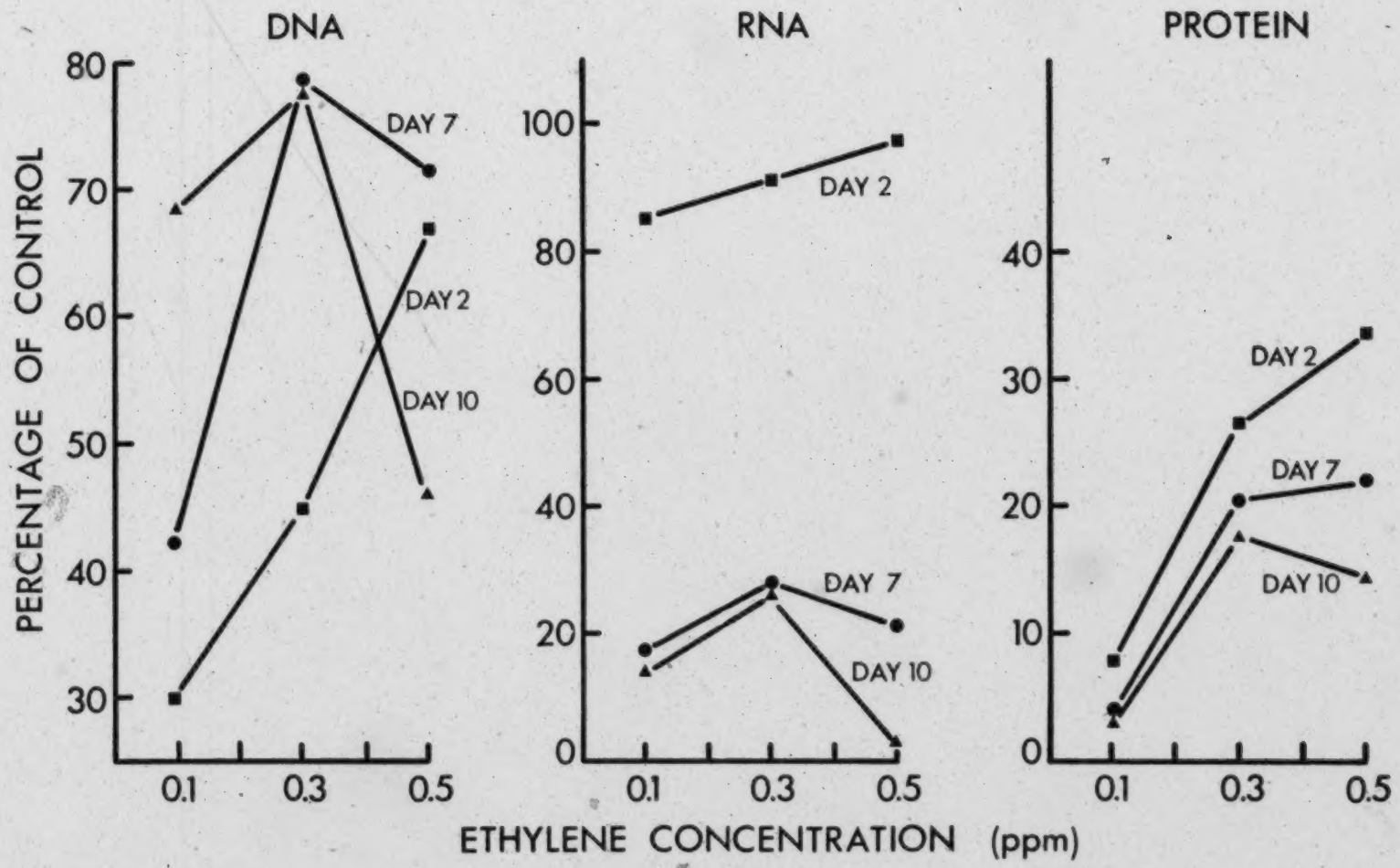
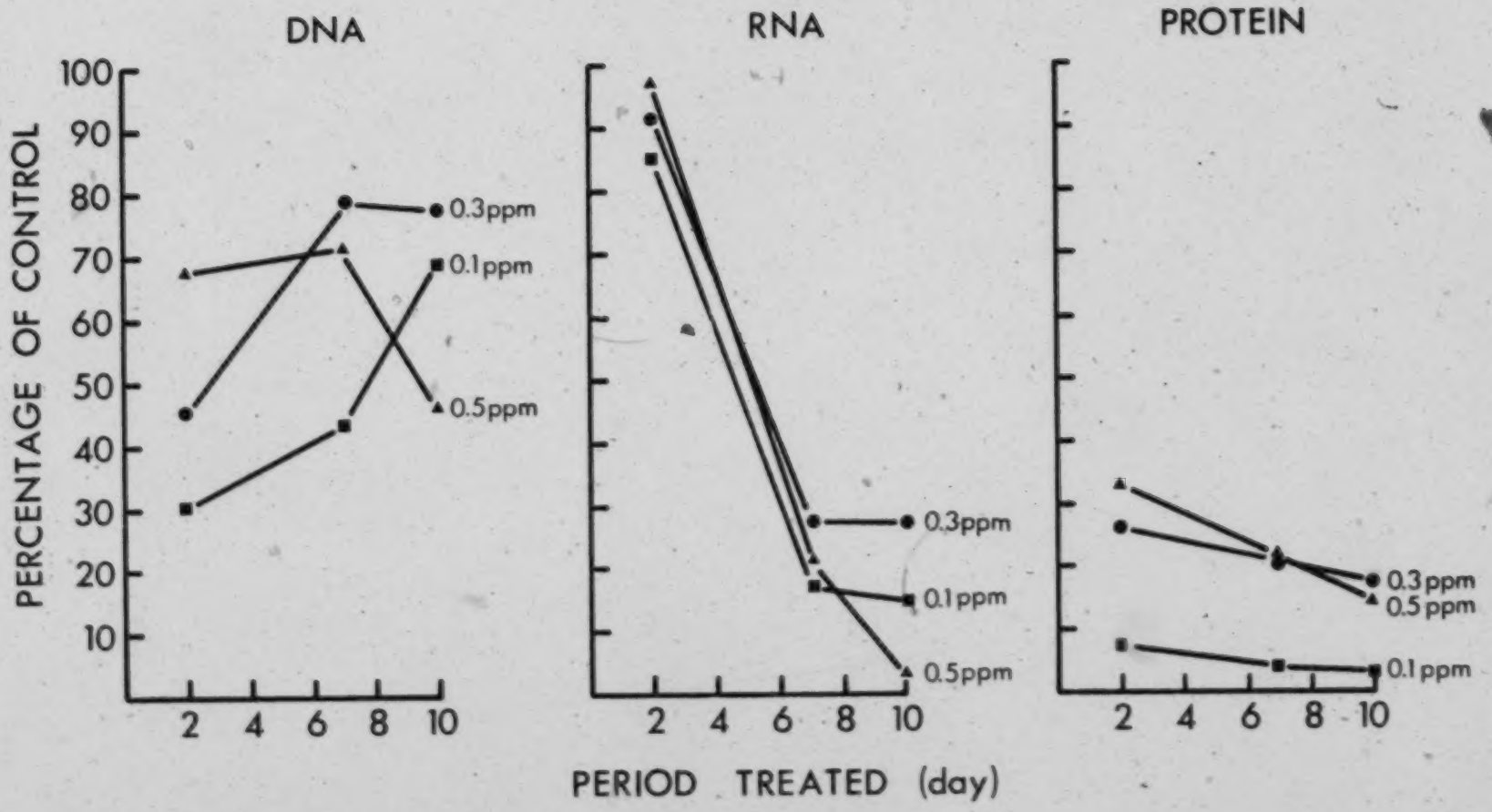

DNA
RNA
PROTEIN
PERCENTAGE OF CONTROL
80
70
60
50
40
30
100
80
60
40
20
0
40
30
20
10
0
DAY 7
DAY 2
DAY 10
DAY 2
DAY 7
DAY 10
DAY 2
DAY 7
DAY 10
0.1
0.3
0.5
ETHYLENE CONCENTRATION (ppm)

FIGURE 24: Effect of Ethylene During the Forcing Period

Tulip plants (cv. Paul Richter) were exposed to varying ethylene concentrations and for different lengths of time.
The figure gives the effect of the length of exposure on the DNA, RNA and protein levels (percentage difference from control).


DNA
RNA
PROTEIN
PERCENTAGE OF CONTROL
100
90
80
70
60
50
40
30
20
10
2
4
6
8
10
0.3ppm
0.1ppm
0.5ppm
PERIOD TREATED (day)

similar to those of high concentrations over a shorter period. Thus the DNA levels of buds exposed to 0.1 ppm ethylene for 10 days gave essentially similar DNA concentrations to the buds exposed for 2 days to 0.5 ppm ethylene. As the stage of growth of the buds was changing during the course of the experiment, the percentage difference from control of DNA was used for comparison. (The percentage difference from control was expressed as:- $\frac{\text{treated-control}}{\text{control}} \times 100$). Percentage difference from control of DNA was 68.22% for the 10-day exposure at 0.1 ppm and 67.14% for that of the 2-day exposure at 0.5 ppm (Table 11).

From the results presented, it is apparent that the threshold concentration of ethylene is around the 0.3 ppm level. In addition, the length of exposure had to be greater than 2 days for visible ethylene effects. This was confirmed by the morphological changes observed. No flower-bud blasting was observed at all ethylene concentrations (0.1, 0.3 and o.5 ppm) when the exposure period was 2 days, however, with the longer exposures blasting was evident at the 0.3 and 0.5 ppm ethylene levels (Figure 7.1). The buds of the plants at 0.3 and 0.5 ppm ethylene concentrations were severly blasted (Figure 16.2) after the 10 day exposure.

b. Effect on RNA

The RNA levels of ethylene treated buds were higher than the levels of the control buds. The RNA content of the buds exposed to ethylene for a 2-day period increased with the increasing ethylene concentrations used (Figures 21 and 23). This increase was proportional to the ethylene concentration (Figure 23). The relationship between RNA and ethylene concentration was no longer manifested with the longer exposures (7 and 10 days). RNA levels increased up to the 0.3 ppm ethylene level for both these exposures and then declined. The decrease, as in DNA, was greater with the 10-day exposure (Figures 21 and 23).

Figures 22 and 24 give the effect of length of ethylene treatment on RNA levels. At all the ethylene concentrations (0.1, 0.3 and 0.5 ppm) employed, RNA levels decreased with increasing exposures of ethylene, however, they remained at higher concentrations than those in the control buds. The decrease was most prominent at the 0.5 ppm ethylene concentration. In fact, the RNA level of the buds after the 10-day exposure at 0.5 ppm ethylene had declined almost to the level of the control; it would have probably declined further with longer exposures to ethylene (Figure 24).

The effect of ethylene on RNA during the early part of the forcing period, showed that the RNA content of the buds was sensitive to low concentrations of ethylene during this period. Thus, it would appear that ethylene may have a possible effect on the RNA metabolism of the buds.

c. Effect on Protein

As with the nucleic acids, protein content in treated buds, were higher than those of the control. In contrast to the nucleic acids, the greatest increase in protein levels were between 0.1 and 0.3 ppm; whereas with the nucleic acids a substantial increase occurred between 0 and 0.1 ppm ethylene (Figures 21 and 23). In addition, protein content was not proportional to the ethylene concentrations after the 2-day exposure, however, the nucleic acids and ethylene concentrations exhibited a linear relationship (Figure 23).

Protein levels of buds (when exposed to varying concentrations of ethylene) decreased with increasing exposure periods. This decrease, as with RNA, was most obvious at the 0.5 ppm ethylene concentration (Figure 21). Thus the effect of exposure period on protein concentration of buds has a closer similarity to its effect on RNA than on DNA, when compared. This relationship between RNA and protein may be explained on the basis of the

sequence of protein synthesis (Figure 11).

d. Summary of the Effects

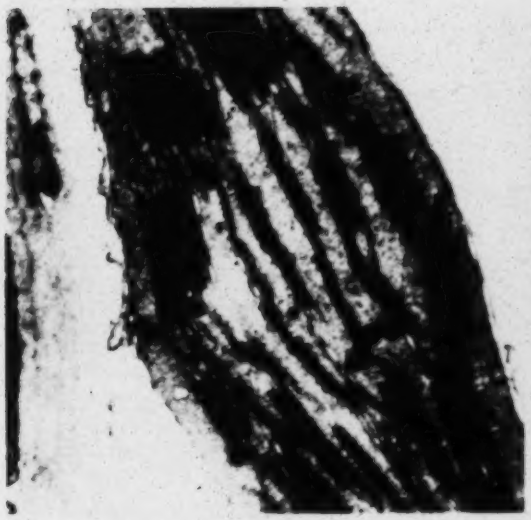
A combination of the results obtained on DNA, RNA and protein suggested that ethylene acts at the site of DNA synthesis. It is also apparent from the results that 0.3 ppm ethylene is the threshold value for direct blasting of flower-buds during this period. A similar threshold value was obtained by Munk (76). The experiment with varying lengths of ethylene treatment indicated that exposures should be longer than 2 days for permanent damage to occur. Apparently plants exposed to ethylene for short (less than 2 days) periods were able to recover from the effects of the gas.

The leaves of plants exposed to ethylene (during the early part of the forcing period) although green, were curled. An ultrastructure study was conducted to examine the possibility of ethylene affecting the chloroplast structure. From the above study on the flower buds, it was evident that irreversible changes occurred after the 10-day exposure. The last leaf of these plants was used in this study. The leaves were fixed in glutaraldehyde and osmium tetroxide, embedded in Araldite, and stained in uranyl acetate and lead citrate.

The electron micrographs of the chloroplast are shown in Figure 25. These micrographs showed that ethylene caused a disruption in the chloroplast structure. The thylakoid membranes appeared to be drawn apart with the increasing ethylene concentrations, hence causing the disintergeration of the grana. This may be caused by the effect of ethylene on the lipid component of the membrane (2). The presence of large osmophilic bodies within the chloroplast structure upholds this view. The disruption in the ultrastructure of the chloroplast may account for the inhibitory effect of ethylene on photosynthesis, even when the gross anatomical

FIGURE 25: Effect of Ethylene During the Forcing Period

Tulip plants (CV. Paul Richter) were exposed for a period of 10 days to 0, 0.1, 0.3 and 0.5 ppm ethylene. The last leaf of the plants were fixed in glutaraldehyde and osmium tetroxide and stained with uranyl acetate and lead citrate.
The electron micrographs show that the chloroplast lose their grana structure with the concentration of of the gas in the atmosphere. The membrane structure of the grana from the 0.5 ppm treatment is almost totally disrupted.

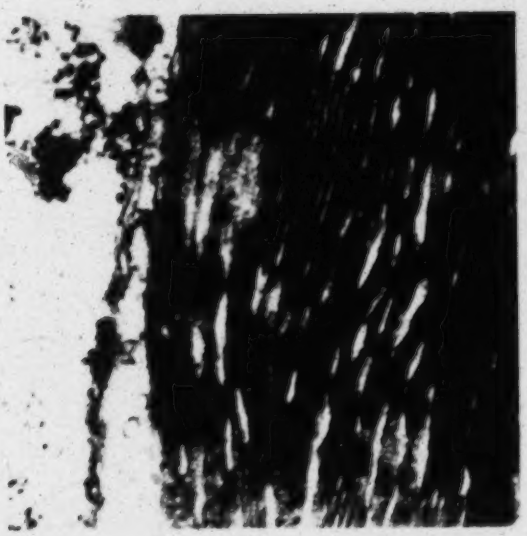


1.

Control

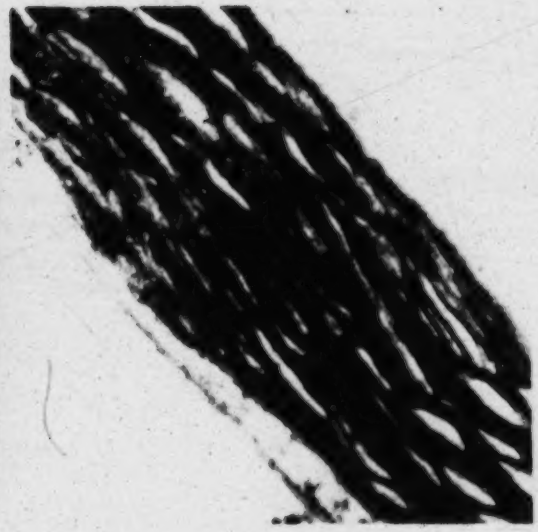
2.

0.1 ppm Ethylene



3.

0.3 ppm Ethylene



4.

0.5 ppm Ethylene

structure of the leaves appear normal.

Once the flowers are in bloom, exposure of the plants to ethylene do not cause severe blasting of the flower-buds. Blasting during this period was very mild. The blasting resembled the ethylene-induced wilting in orchid flowers (31) and sleepiness in carnations (103). Topple, (87) also called 'wet stem' or water neck' (flower stem collapses and falls over), was also evident in the treated flowers.

The apparent resistance of the flower to ethylene during this period may be due to the innate growth habit of the plant. The reproductive organs of plants have been shown to be efficient producers of ethylene (80, 99). Pollen has been shown to produce sufficient amounts of ethylene to cause the fading of Vanda orchid blossoms (23).

During this stage of growth, ethylene did not cause severe blasting of the flower-buds; nevertheless, the number of daughter bulbs produced was affected. Although, the number of bulbs produced by the ethylene treated plants increased with increasing ethylene concentrations, the size of these bulbs was reduced. Table 12 gives the effect of ethylene concentrations on the number of bulbs formed. Similar results have been reported in the case of ethylene treated onion plants (56), and also in the tuberization of Solanum tuberosum (25) and dahlias (13). Ethylene was shown to cause the swelling of the leaf bases of treated plants. Subsequent to the swelling, a greater amount of the assimilates were conveyed to these swellings. However, the transport of the assimilates to these areas is inhibited by ethylene. It has been shown (with the potato) that prolonged exposures to ethylene after the initiation of the tuber initials do not lead to tuber formation - the swellings do not accumulate starch (44, 60). The relatively high concentrations of assimilates at these localized areas probably encouraged cell division and cell ezpansion, thus

TABLE 12: Effect of Ethylene During the Forcing Period (Day Temp. 18 C, Night Temp. 16 C)

Ethylene Concentration (ppm)	Number of Bulbs Formed
0	3
1	3
2	5
5	7
10	7

Tulip (cv. Paul Richter) plants treated during forcing after the flowers have bloomed. The plants were exposed to ethylene concentrations of 0, 1, 2, 5 and 10 ppm for a period of one week. The ethylene was then removed and the plants allowed to mature. After the foliage leaves had died down, the bulbs were lifted and the number of bulbs formed noted.

leading to the formation of a larger number of bulbs. The reduced size of the bulbs is apparently caused by the higher demands on the assimilates than that normally encountered by the plant.

The greatest rate of new bulb growth occurs after the blooming of the flowers (71). Hence, if nutrients are supplied at appropriate levels subsequent to the ethylene treatment (i.e. when flowers are in bloom), it could probably increase the size of the bulbs produced by treated plants.

V. GENERAL DISCUSSION

This study showed that the susceptibility of tulips to ethylene varied with the cultivar as well as the satge of growth of the plant tissue.

The susceptibility of cultivars to ethylene is judged on their response to:-

1. ethylene concentration,
2. duration of ethylene exposure.

In this study, gummosis and gum blisters did not occur on the bulbs of the Paul Richter cultivar even when they were exposed to 100 ppm ethylene concentrations for one week; while with the Darwin 33 cultivar the disorder was apparent after a week of exposure at 1 ppm. Both these cultivars are Darwin tulips, however, they vary in their susceptibility to ethylene. There is evidence in the literature that several other cultivars vary in their reaction to ethylene; not all cultivars react by the production of gum blisters (52).

Gum blisters, when formed, were limited only to the outermost bud-scale leaf. It would prove worthwhile to examine the structure of the cells below and around the gum blisters to note the changes that occurred. An analysis of the gum would also prove interesting. If this exudate is in any way related to cell sap, ethylene may have altered the permeability of the cell membrane, thus permitting the excretion of the 'gum'. In this case, however, the excretion would be passive. Although ethylene has been shown to affect cell permeability (93), the possibility of gummosis being an active process should not be ignored.

In this study, no bud necrosis occurred, however, open buds were formed. This was probably due to the absence of mites (74). The exact

processes involved in the formation of bud necrosis are still unknown. It is thought that the complex of mites, bacteria and moulds bring about the decay associated with necrosis (Figure 15). Thus, it may be possible that the mites function as transporting agents, bringing the bacteria and moulds into contact with the cells. The direct agent for bud necrosis may be resolved if it were possible to separate the mites from the micro-organisms (production of sterile mites). Then if necrosis is produced from these 'sterile' mites it may be concluded that mites are the immediate agents in bud necrosis. Nevertheless there are no known means of separating mites from bacteria.

It has been suggested that ethylene functions as a morphogenetic agent, altering the morphology of the bud. Vegetative:reproductive and femaleness:maleness is governed by the ratio of growth promoter to growth inhibitor (88) and as ethylene may function as either, it may alter the morphology of the plant. In addition to the above it has been suggested (74) that ethylene may also change the composition and metabolism of the stamens that they become more attractive to mites and micro-organisms.

The effect of ethylene on nucleic acid and protein content of the buds showed an increase with increasing concentrations of ethylene, when bulbs were treated during the postharvest maturation period (Figure 8). An examination of the DNA:RNA and DNA:protein ratios of the buds showed that the ratios increased with increasing ethylene concentrations. The increase in the DNA levels relative to RNA and protein would imply that cell division was probably taking place. The protrusion of the stamens above the tepals showed that ethylene may have either inhibited the growth of the tepals or promoted the growth of the stamens. The change in the relative growth rate would give rise to open buds.

After the bulbs had been planted and cooled, the shoots

emerging from ethylene-treated bulbs were shorter than the controls. The higher the concentrations of ethylene during the exposure, the shorter the shoots. When these bulbs reached the flowering stage, it was found that the basal internodes were shorter and the leaves narrower than normal. The higher the ethylene concentration the greater the differences. Difference in the total length of the stem and length of the leaves and tepals showed no correlation with prior ethylene treatments.

Thus, it was concluded that bulbs exposed shortly after lifting can resume their growth during subsequent storage and after planting. However, the influence of early exposure to ethylene could be seen in dissected buds at the end of the storage period (open bud). Plants developing from exposed buds were generally more tenuous.

Buds exposed to ethylene during the cooling period were generally less responsive to the gas. No abnormalities occurred from bulbs exposed to ethylene during this period. The nucleic acid and protein content of the buds, however, were affected; indicating that the metabolism of the bud was affected. When bulbs were exposed to 0, 5 and 10 ppm ethylene for 4 weeks and the gas removed, the RNA:protein ratio of the treated buds and that of the control buds approached a common value at some period after the removal of the ethylene (Figure 18). This ability of the bud to revert back to similar proportions of RNA and protein, as that in the control, could explain the lack of disturbance in the flower structures.

The effect of ethylene on tulips during the forcing period revealed that the susceptibility of the flower to the gas depended on the time of the ethylene exposure. The flower was most sensitive to ethylene during the early part of the forcing period, before the last foliage leaf had uncurled to reveal the bud. However, once the flower blossomed it was relatively insensitive to the gas and only very mild forms of blasting

occurred when the treatment was during this period. This observation is in agreement with that made by Munk (76).

An examination of the effect of ethylene during the different growth stages of the bulb, revealed that the effects of ethylene were most pronounced when the metabolic activity of the plant was high (during the period of flower initiation and formation, and the early part of the forcing period). During high metabolic activity the rate of turnover of the products is high. Thus a slight alteration in a step in the metabolic sequence could upset the whole metabolism of the plant.

There was substantial evidence in this study that ethylene may act at the level of the gene. If such is the case production of several enzymes in response to ethylene might suggest initiation of transcription of an operon - the structural genes which give rise to mRNAs for several enzymes (94). This then would help to explain the DNA-RNA-protein relationships observed.

Increase synthesis of DNA could mean either increased rate of cell division or the production of polyploids. The former may have occurred during ethylene treatment of the bulbs at the postharvest maturation period. Ethylene treatment during this period altered the number of flower parts. The increase in DNA levels observed in buds treated during the early forcing period may have been due to the formation of polyploids - duplication of chromosomes without cell division. The formation of polyploids could lead to non-viable cells; this then could explain the direct blasting of the flower-buds observed in treatments during this period. It would prove interesting to study the ultrastructure of the flower-buds (as affected by ethylene) during these two (postharvest maturation and forcing) periods.

In summary, it may be stated that ethylene functions in a variety of ways. Similar actions of ethylene on different structures or

plants can give rise to different results. When considering the effects of ethylene all conditions have to be standardized, even slight alterations in procedure may cause significant changes in the results. The growth stage of the plant is very important, plants apparently respond differently to ethylene treatment during different growth stages.

This study showed too that tulips are very sensitive to ethylene. This is contrary to existing (20) literature which states that monocotyledonous plants are rather insensitive to ethylene.

The tulip appears to be a good tool for the study of the effects of ethylene because it is very sensitive to the gas. However, it is not possible to predict the developmental stage of the bulb without dissecting it. This fact renders the use of whole bulbs somewhat cumbersome and inaccurate. For future biochemical work we would suggest the use of embryos maintained and grown on artificial culture media.

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